THE CHANGING SPATIAL EPIDEMIOLOGY OF MALARIA IN THE DEMOCRATIC REPUBLIC OF THE CONGO

Molly Deutsch-Feldman

A dissertation submitted to the faculty at the University of North Carolina at Chapel Hill in partial fulfillment of the requirements for the degree of Doctor of Philosophy in the Department of Epidemiology.

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Approved by:

Steven Meshnick

Jessie Edwards

Michael Emch

Emily Gower

Jonathan Juliano

Robert Verity

ABSTRACT

Molly Deutsch-Feldman: The Changing Spatial Epidemiology of Malaria in the Democratic Republic of the Congo
(Under the direction of Steven R. Meshnick and Emily W. Gower)

Malaria remains a significant public health problem worldwide, and especially in the Democratic Republic of the Congo (DRC), where approximately 12% of global cases occur. Despite this burden, very few national malaria studies have been conducted in the DRC, particularly amongst adults. Because of this, critical questions, such who is at highest risk and where transmission is highest, remain under-studied. This information is needed to tailor interventions to individuals and areas in which they will be most effective. We aimed to fill this gap in the literature using data from the population based, nationally-representative Demographic and Health Surveys (DHS) conducted in the DRC.

Using DHS data, we evaluated the status of malaria prevalence amongst adults in the DRC, and determine changes in prevalence over time. We found that the national prevalence is high, approximately 30%, and that the prevalence of patent infections increased from 2.4% in 2007 to 7.5% in 2013. We identified several risk factors for infection, such as traditional housing and decreased within-household net coverage. However, we also found that while uptake of malaria interventions has increased since 2007, use of long-lasting insecticide treated nets (LLIN) and intermittent preventative therapy during pregnancy both remain low. Increasing LLIN use was associated with only a small reduction in prevalence of patent infection, pointing to the need to re-evaluate current malaria control strategies.

Overall, this dissertation highlights the high prevalence of infections amongst adults and the need for massively scaled up malaria control efforts. The findings identify individuals and areas that most need attention. These findings will help the DRC Ministry of Health plan future malaria control programs and help ensure that such programs are maximally effective. This dissertation also underscore the importance of studying malaria amongst individuals of all ages.

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ACCM	all-cause child mortality
CAR	conditional auto-regressive
DIC	deviance information criteria
DHS	Demographic and Health Survey
DRC	Democratic Republic of the Congo
ITN	insecticide treated nets
IPTi	Intermittent preventative therapy for infants
IPTp	Intermittent preventative therapy during pregnancy
IRS	Indoor residual spraying
LLIN	long-lasting insecticide treated net
LOD	limit of detection
MAP	Malaria Atlas Project
MIP	molecular inversion probe
MOH	Ministry of Health
PCA	principal components analysis
PCR	polymerase chain reaction
PMI	President's Malaria Initiative
RDT	rapid diagnostic test
SANRU	Santé Rural
SNP	single nucleotide polymorphism
SP	Sulfadoxine/pyrimethamine
SVC	spatially varying coefficients

WHO	World Health Organization

CHAPTER ONE: SPECIFIC AIMS:

Malaria remains a significant public health problem in the Democratic Republic of the Congo (DRC), with an estimated 25 million cases in 2018[1]. Despite this burden, there have been very few national malaria studies conducted in the DRC, particularly amongst adults. Because of this, critical questions, such as who is at highest risk and where transmission is highest, remain under-studied. This information is needed to tailor interventions to individuals and areas in which they will be most effective. The objective of this study is to answer these questions using nationally representative data.

A comprehensive multi-level risk factor analysis for malaria infection in the DRC is needed in order to identify individuals who are at a higher risk for infection. No group has assessed risk factors for infection amongst adults in the DRC since the 2007. Several recent studies have demonstrated associations between various community-level factors and malaria risk amongst children in the DRC[2,3]. Thus, determining larger scale risk factors is critical for understanding malaria epidemiology, and an important part of creating intervention policies. Additionally, as several studies from across the world have demonstrated that malaria transmission dynamics differ between urban and rural areas, comparing risk factors between the two settings is another important element of targeting prevention efforts to be most effective[4].

Past work from the DRC has demonstrated the heterogeneous spatial epidemiology of malaria across the country, adding an additional complexity to prevention efforts. A study of adults in the DRC from 2007 demonstrated that malaria prevalence ranged from 0-82% depending on the region[5]. Additionally, while preliminary data from the Demographic and

Health Surveys (DHS) Program indicate that the national prevalence of malaria amongst adults remained steady from 2007 to 2013, the longitudinal differences in prevalence were highly variable across the country. Some provinces experienced a drop in prevalence since 2007 while others exhibited an increase. Understanding how and why these patterns of transmission are changing over time is necessary for identifying regions most in need of interventions. No studies have comprehensively investigated how the geographic distribution of malaria infection amongst adults has changed since in the past decade or linked these changes in malaria prevalence with intervention implementation.

To address these gaps in knowledge we have formulated two Specific Aims for this study:

Specific Aim 1: A) Determine individual and cluster-level risk factors for *Plasmodium falciparum* infection amongst adults living in the DRC and B) evaluate whether the effects of these risk factors are modified by urban/rural settings. Data will be derived from the 2013 DHS. *Plasmodium falciparum* infection will be determined using a *pfldh* PCR assay. We will use the DHS data to build a multi-level model and estimate individual and community-level risk factors such as education or wealth index. Additionally, we will include the cluster-level proportion of drug resistant infections, determined by genetic sequencing, as a potential risk factor. Risk factors identified by this model will then be further assessed for modification by comparing strata specific risk estimates for both urban and rural areas (as designated in the DHS).

Specific Aim 2: A) Compare the spatial distribution of patent *Plasmodium falciparum* malaria between 2007 and 2013 amongst adults in the DRC B) Assess the effects of community-level interventions on changes in patent malaria prevalence. Using data from the

both the 2007 and the 2013 DHS, we will compare the prevalence and spatial distribution of patent *Plasmodium falciparum* infections (at least 100 parasites per uL of blood) amongst adults over time. We will create risk maps to identify whether province level prevalence increased or decreased between 2007 and 2013. Additionally, we will determine the effect of two malaria interventions, insecticide treated bed-nets and intermittent preventative therapy during pregnancy, with increasing or decreasing malaria prevalence between the two survey time points using geospatial Bayesian modeling.

This proposal is innovative as it utilizes epidemiologic, genetic, and spatial data to determine risk factors for malaria infection. Additionally, it is the first study, to our knowledge, to evaluate longitudinal changes in national malaria prevalence amongst adults in the DRC, and to link these changes to specific interventions.

The findings from this study will directly aid the DRC in its efforts to reduce malaria transmission; it will also have implications for other sub-Saharan African countries. Identifying risk factors for malaria infection will help direct intervention efforts to individuals at highest risk of infection. Understanding the heterogeneous spatial epidemiology of malaria within the DRC will help identify areas in which malaria transmission has increased over the past decade. Lastly, linking changes in malaria prevalence with interventions will help determine the effects of these interventions on malaria prevalence over the past decade.

CHAPTER TWO: BACKGROUND AND INNOVATION

Malaria Overview:

Malarial disease is caused by infection with the *Plasmodium* parasite, transmitted to humans through a bite from an infected female *Anopheles* mosquito. There are five *Plasmodia* species known to infect humans*: P. falciparum, P. vivax, P. ovale, P. malariae,* and *P. knowlesi.* Though most infections worldwide are caused by *P. vivax*, *P. falciparum* is responsible for the most deaths[6,7]. Symptoms usually manifest within 7-30 days after an infectious bite[6]. Typical symptoms of uncomplicated malaria include fever, chills, anemia and an enlarged spleen. However, some cases proceed to complicated malaria, which includes cerebral malaria and severe anemia[6]. Pregnant women can also develop placental malaria, potentially leading to premature delivery or a low birth weight baby[6,8]. Infections are treatable, although the available treatments depend on specific country policies.

There are several methods for diagnosing malaria; the diagnostic of choice is largely dependent on context, cost, and feasibility. These methods are: rapid diagnostic tests (RDT), microscopy, clinical diagnosis, and polymerase-chain reaction (PCR). RDTs are point of care tests that only require a single drop of blood for diagnosis. The tests detect the presence of malaria antigens (most often the histidine-rich protein II antigen) in the blood and return a positive or negative result is about 15 minutes. Microscopy, considered the gold standard, requires a drop of blood to create a thick-smear microscope slide that is then read by a trained microscopist to determine the presence of parasites. Clinical diagnosis, as the name implies, is determined after a clinical evaluation in which a patient is assessed for the presence of typical

malaria symptoms such as fever, anemia, or an enlarged spleen. PCR is a laboratory based method that detects Plasmodium DNA from a patient blood sample. Though the most sensitive diagnostic, PCR requires specialized equipment and laboratory materials[9]. Each method presents pros and cons; the purpose of the diagnostic data and available resources often dictate which method will be used.

General risk factors for malaria disease include both biological and behavioral factors. Biological factors that influence risk include: younger age, human genetic factors (such as sickle cell trait), or previous infection[10]. Behavioral/societal influences include: lower income, agricultural exposure, and lack of proper housing[10]. While these factors are generally considered to be risk factors for disease, the relative effects of each of these may differ across populations. Studies from specific regions, as will be discussed further in the context of the Democratic Republic of the Congo, have also identified more individual and community-level risk factors[2,3,5]. Thus, findings from malaria studies tend to be specific to the specific context of study.

The Global Burden of Malaria:

Malaria remains a major public health problem worldwide. In 2018, there were approximately 228 million cases and 405,000 deaths due to malaria[11]. The global distribution of cases in 2018 demonstrates the wide geographic spread of malaria[12,13] (**Figure 2.1**). Over 3 billion people live in malaria endemic countries and are at risk for infection[13]. While the World Health Organization (WHO) reported an increase in the number of cases between 2015 and

2017, the estimates from 2018 indicate that the number of cases globally has since dropped slightly (228 million cases in 2018 vs. 231 million in 2017)[11,14].

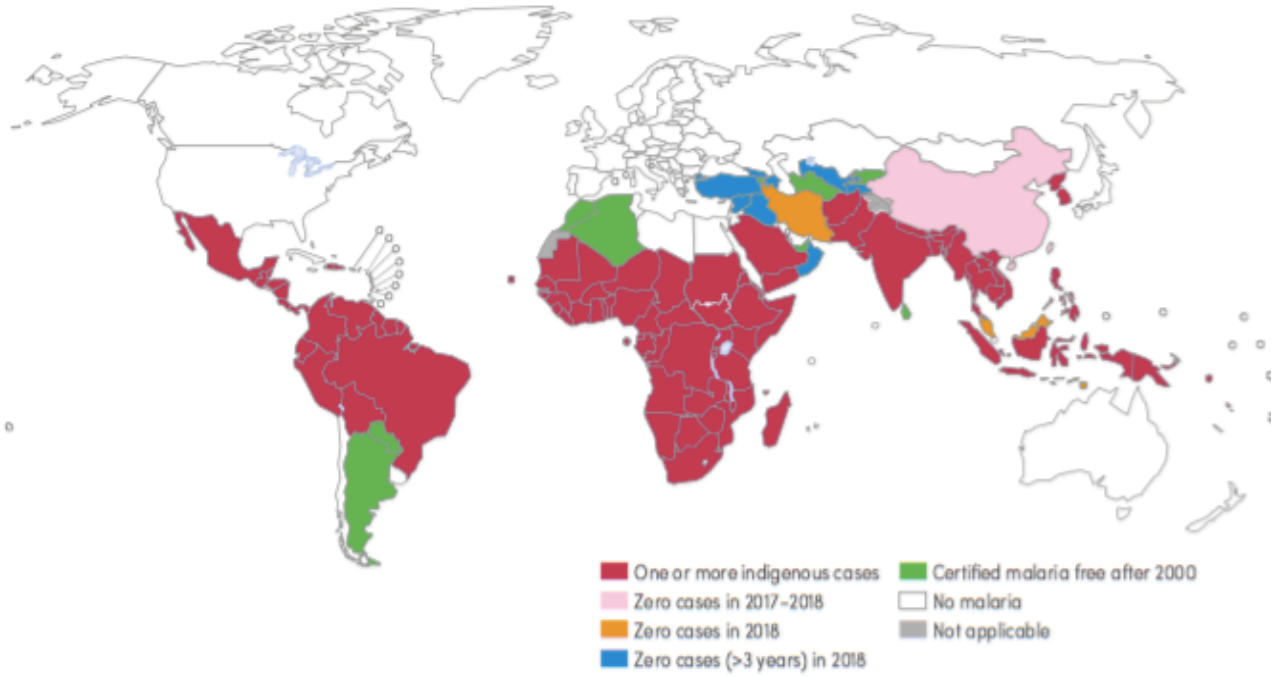


Figure 2.1: 2018 global distribution of malaria worldwide. Countries in red reported at least one malaria case. Countries in yellow and blue reported zero cases in the past year (yellow) or previous three years (blue)[11].

Since 2000, with the creation of the Millennium Development Goals, there has been a significant reduction in malaria transmission worldwide and in Africa particularly[15]. In 2015, the Malaria Atlas Project (MAP), a consortium of researchers based at Oxford University, published a series of maps depicting the changes in malaria prevalence (detect by RDT) amongst children across Africa between 2000 and 2015[16]. The maps, generated using a spatial-temporal Bayesian geostatistical model, indicate a steep drop in prevalence in many regions across the continent[16] (**Figure 2.2**). However, they also highlight remaining hotspots of malaria in West and Central

Africa[16]. MAP's work highlights the enormous progress in malaria control over the past fifteen years, and identifies regions that will still need attention in the coming years.

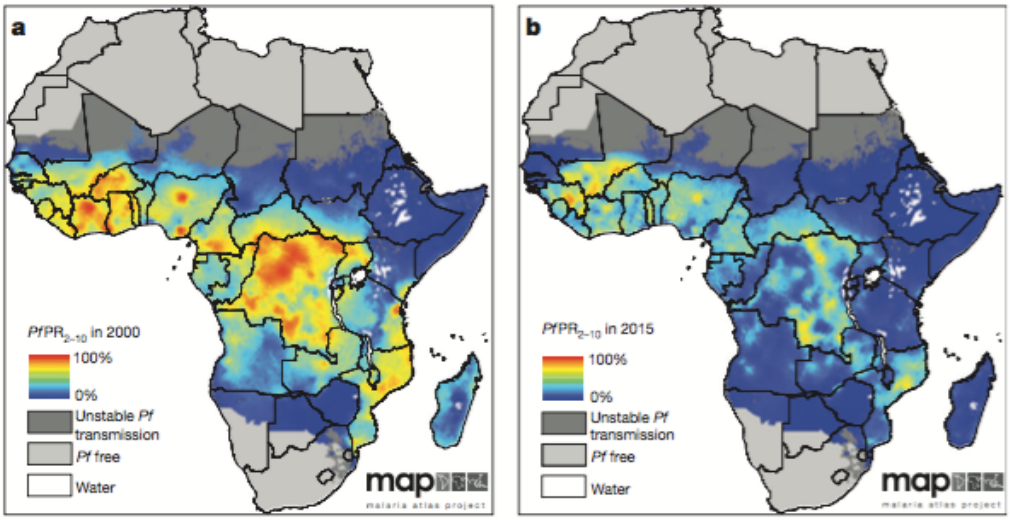


Figure 2.2: Estimated changes in malaria prevalence (as detected using RDTs) between 2000-2015 amongst children ages 2-10[16].

The success of malaria control programs since 2000 are in large part due to significant increases in international investment. In the past two decades, there has been a nearly fifteen-fold increase in international spending on malaria control programs as well as research and development[15,17]. Nearly 2 billion dollars were invested in malaria programs in the WHO Africa region in 2016, an increase from 960 million per year over the past decade[18]. In the next decade, a further scale up of investment is planned to help more countries achieve elimination[19]. Thus, despite the recent uptick of cases, international attention and effort is focused on reducing malaria burden.

Malaria Interventions:

There are several methods for preventing malaria infection, each of which acts through a different mechanism to stop the spread of infection. Malaria interventions can be categorized into

three main groups: insecticide treated nets (ITNs), indoor residual spraying (IRS), and chemoprevention[20]. ITNs work in two ways, providing a physical barrier between an individual and the mosquito to prevent biting and by killing the mosquito with insecticide[21,22]. Many nets used today, called long lasting insecticide treated nets (LLINs), are specifically engineered to have extended insecticide efficacy[22,23]. The second option, IRS, involves spraying the inside of a building with insecticide to kill mosquitos[24]. This method targets a subset of *Anopheles* species that rest inside after taking a blood meal[25]**.** Lastly, many countries use chemoprevention methods by providing individuals, usually vulnerable groups such as pregnant women or children, with anti-malarial medication to prevent infection. Intermittent preventative therapy during pregnancy (IPTp) with Sulfadoxine-pyrimethamine (SP) is recommended by the WHO in areas of moderate to high transmission[26,27]. IPTp consists of providing all pregnant women with three doses of SP regardless of whether the woman is infected. Similarly, intermittent preventative therapy for infants (IPTi) consists of providing infants with three doses of SP within the first year of life regardless of infection status[28]. Some countries also implement seasonal chemoprevention during months of high transmission[29]. For this, WHO recommends using SP and Amodiaquine[29]. Many studies have assessed the efficacy of these interventions, demonstrating a range of protective effects across various geographic areas and transmission settings[16,30,31]. The choice of which interventions to use depends largely on the specific entomological and epidemiological environment, as well as available resources.

Over the past two decades, researchers from across the world developed RTS,S, the first malaria vaccine shown to be protective against *P. falciparum* infection[32]. Phase 3 clinical trials for the vaccine concluded in 2014 and demonstrated that despite incomplete protection, there is still a net public health benefit of using the vaccine[33,34]. In 2017, WHO began a pilot program to

implement the vaccine in three countries (Kenya, Ghana, and Malawi) for children under the age of 2 years old[32,35]. As this program progresses, we will learn more about the effectiveness of this vaccine and its potential to help reduce transmission worldwide. Additional on-going research is investigating alternative vaccines such as those that block transmission of the malaria parasite rather than preventing infection[36,37]. These vaccines are still in development but are a promising avenue of research.

Though these various intervention methods have all been shown to be generally protective against malaria, new threats to intervention efforts are spreading. First among these is resistance to many commonly used anti-malarial drugs including SP (the drug used for IPTp and IPTi). The history of malaria control is marked by the introduction of novel therapeutics, followed quickly by the development of resistance[38]. A recently completed study from our group using a subset of samples from children from the 2013 DRC Demographic and Health Survey estimated that 76% of the children with malaria had an infection with least one of the mutations shown to be linked with SP resistance[39]. Similarly, resistance to insecticides has led to decreased efficacy of LLINs and IRS[40,41]. Mutations of the mosquito genome block the insecticidal activity of the chemicals used to treat nets or used in IRS[40]. Though studies have shown that treated nets are still more protective than untreated nets in the presence of resistant mosquitos, rising resistance may seriously impact malaria control efforts[41]. Other roadblocks for prevention efforts are non-use and misuse of LLINs. A study from Ethiopia found that while 91% of survey respondents reported owning at least one net, only 65% reported that they had slept under the net the previous night[42]. The study also found a small number of communities in which nets were being used for purposes such as curtains, table cloths, or for farming[42].These findings are

concerning and indicate the need to monitor the effect of various interventions in preventing transmission.

Spatial Epidemiology of Malaria:

Like other infectious diseases, malaria exhibits a complex spatial epidemiology, with transmission intensity differing across regions[43]. Countries are often categorized based on the level of transmission, for example: sustained year-long transmission, seasonal transmission, or low transmission[43]. However, these categorizations often mask within country heterogeneity. Many countries have some regions of high transmission and others of seasonal or low transmission[5,44]. In certain countries, this "micro-geographic" variation is observed even with a single city[45]. These heterogeneous dynamics are a result of various factors including differing vector populations across the country, ecological environments, and population density[4,46–48]. Different epidemiologic profiles within a single country indicate the need to study malaria on sub-national spatial scale.

Spatial patterns of malaria transmission are in part driven by human mobility patterns. Infected persons can introduce malaria to new areas by traveling from an endemic region to a non-endemic region[49]. A study from Kenya used mobile phone data to identify specific "source" areas of high transmission from which people travel to "sink" areas of low transmission[49]. Work from Zambia demonstrated that individuals move to and from areas of high transmission even on a daily basis[50]. These findings complicate malaria control efforts, as human mobility can be difficult to monitor and is not an avenue for easily implementing interventions.

Previous studies have investigated the spatial dynamics of malaria by examining parasite population structure, i.e.: is there evidence that parasite populations are made up of smaller, genetically distinct sub-populations? If so, how are these populations distributed over space?

Understanding population structure can help shed light on important questions, such as the degree of local versus national transmission. Recent studies have used genetic sequencing data to demonstrate that such structure does exist amongst *P. falciparum* populations in Southeast Asia[51,52]. The extent of this structure in sub-Saharan Africa is an area of active research as novel genetic and analytical tools are further developed[53].

Mapping of malaria prevalence across the world has become a hotbed of research as new statistical methods are developed. Though maps of malaria have existed for many decades, modern computing power has allowed the development of new geostatistical models, particularly those that use a Bayesian framework[16,54–56]. These models, used by groups such as MAP, have deepened our understanding of malaria epidemiology and highlighted avenues for future development[16,55]. Additional methods seek to link multiple types of data, such as parasite genetic sequencing data and cellphone records, to more accurately describe and predict local transmission patterns[54].

Urbanization and Malaria:

Another element of the spatial epidemiology of malaria is the effect of urbanization. The growth of urbanization over the past century has helped contribute to the drop in malaria transmission worldwide, with one study from Hay et al estimating that urbanization lead to a 6.7% reduction in deaths due to malaria[57,58]. Though many infectious diseases thrive in urban areas due to high population density, malaria prevalence is often much lower in cities than in rural areas[59,60]. This is largely a result of reduced vector populations in cities and lower biting rates. Better access to therapeutics, both for prevention and treatment, also contribute to lower transmission[59]. The drop in transmission within urban centers has largely shifted the burden of malaria to rural areas, a shift that has important implications for malaria control efforts[4]. Cities

are vastly different from rural areas in terms of the ecological landscape and demographics, both of which contribute to malaria risk[61]. However, urban malaria should not be discounted as transmission does occur within cities[60]. The differing epidemiology of malaria between urban and rural areas indicates that malaria control efforts must be tailored to specific settings.

The differences between urban and rural areas have also been shown to affect malaria control programs. A study from Myanmar demonstrated that ITN ownership and usage was higher amongst rural populations than urban[62]. Past work conducted in Liberia determined that the effects of bed-nets differed between the settings, with increasing community-level bed-net coverage being protective in urban areas but displaying no effect in rural areas[63]. The authors suggest that this difference could be due to a few reasons including: higher population density in urban areas (leading to increased density of nets), insecticide resistance in rural areas, or insufficient community-level LLIN use in rural areas[63]. Other studies from across sub-Saharan Africa have demonstrated that the protective effect of nets is lower in areas of higher transmission intensity, which is often true of rural areas[64,65]. These findings provide further evidence that control programs must be specific to each area as the effects of various interventions may differ by setting.

Malaria in the DRC:

The burden of malaria in the DRC is one of the highest in the world, with an estimated 25 million cases in 2017[66]. This represents over 10% of cases worldwide[1]. Transmission is characterized as holoendemic, indicating stable year-round transmission in 97% of the country[20,67]. The predominant parasite species is *Plasmodium falciparum*. Transmission is sustained by three Anopheles mosquito species: *A. funestus*, *A. gambiae*, and *A. coluzzi*[67].

Though malaria is often considered a pediatric disease, adult cases remain a significant concern in the DRC. Estimates from the 2007 DHS indicate that the prevalence of infections amongst adults reached as high as 82% in certain regions of the DRC[5] **(Figure 2.3)**. These infections are especially important as most adults, particularly in countries of high transmission such as the DRC, do not exhibit symptoms[68]. Because of this, many of these infections go undiagnosed and untreated, remaining in the population and potentially capable of transmitting to others[68,69]. Additionally, though initially asymptomatic, there is evidence of long term health problems associated with such infections, including anemia, susceptibility to other infections, and cognitive damage[70]. Thus, adult cases are concerning in and of themselves, and must be addressed to move towards elimination.

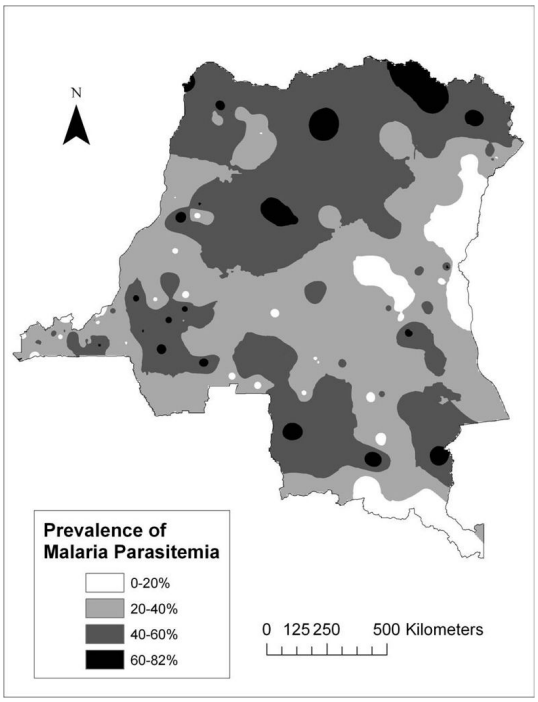


Figure 2.3: PCR-detectable malaria prevalence estimates from 2007 amongst adults in the DRC[5]. Areas of darker color indicate areas of higher prevalence; estimates range from 0 to 82%.

Findings from genetic studies indicates that transmission within the DRC is complex and highly heterogeneous. The DRC, the second largest country in Africa, is considered a "melting-pot" of malaria transmission, connecting East, West, and Central Africa[71–73]. Previous analyses have shown that parasites from the DRC are genetically similar to strains from both West and East Africa, indicating transmission between the DRC and its neighboring countries[71,73]. Additionally, the evidence regarding parasite population sub-structure in the DRC is mixed. Some studies have found no evidence of structure[71,72], though others suggest that drug resistant parasites cluster together spatially[39,53]. Thus, the details of local transmission dynamics remain unclear. However, the overall picture is one of a complex environment of high transmission.

Like many other countries, the spatial distribution of adult malaria infections varies across the DRC. The country is divided into four epidemiologic zones: a mountainous region

with a very short transmission season, a tropical zone with high transmission during the rainy season, an equatorial region with high year-round transmission, and an urban zone with heterogeneous transmission (includes only the capital city of Kinshasa) (**Figure 2.4**)[20]. As stated, prevalence estimates from 2007 range across regions from as high as 82% to as low as 0%[5] (**Figure 2.3**). Additionally, there is evidence of heterogeneity between urban and rural areas, as the prevalence of infection amongst children living in rural areas was found to be higher than that amongst children in urban areas[2]. These dramatic differences make it more difficult to plan intervention programs, as different regions have vastly different needs. Understanding the drivers of these spatial differences is a key element of reducing transmission.

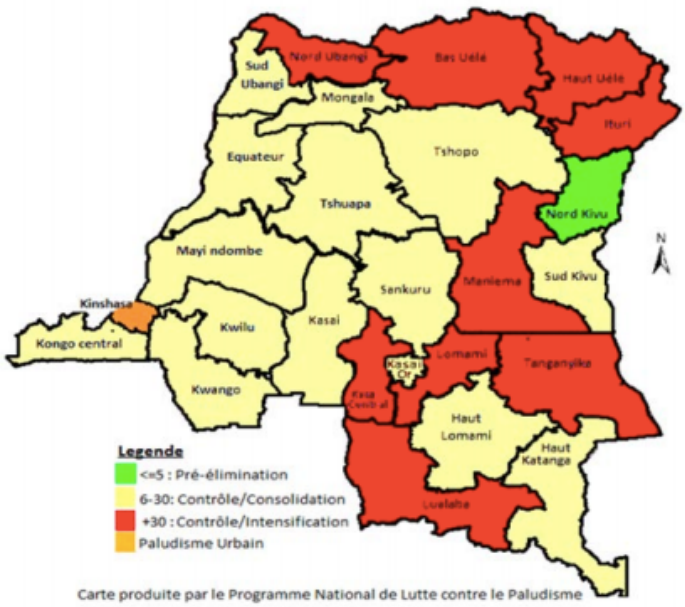


Figure 2.4: Map of epidemiologic zones in the DRC: the mountain region with a short transmission season (green), tropical zone with high transmission during the rainy season (red), the equatorial region with high year-round transmission (tan), and urban malaria, with variable transmission (orange)[20].

Risk factors for malaria in the DRC:

Given the high burden of malaria, determining risk factors for infection is critical for identifying individuals or groups more likely to become infected. Several studies have performed such analyses in the DRC, identifying both individual and community-level risk factors for infection (**Table 2.1**) [2,5,73]. The most recent analysis of adults was conducted using data from the 2007 DHS; multiple analyses of children have been conducted more recently using data from the 2013 DHS.

Table 2.1: Previously identified risk factors for malaria in the DRC

Level	***Risk Factor***	***Association***	***Group studied***	***Study year***
Individual	Age	Older age is protective	Adults and children	2007[5], 2011[74], 2013[3]
	Wealth	Increasing individual wealth is protective	Adults	2007[5]
	Female Sex	Found to be both protective (adults) and harmful (children)	Adults and children	2007[5,73], 2013[3]
	Recent Fever	Recent fever associated with increased risk	Children	2007[73], 2011[74]
	Receiving anti-malarial	Receiving anti-malaria during last pregnancy is protective	Adults	2007[73]
	Bed-net use	Individual bed-net use is protective	Adults and children	2007[5], 2013[2]
Community	Wealth	Increasing community wealth is protective against infection in general, found to be associated with increased prevalence of drug resistant infections	Adults and children	2007[5], 2013[39]
	Education	Increasing community education associated with increased prevalence of drug resistant infection	Adults and children	2007, 2013[39]
	Bed-net use	Increasing community bed-net use is protective	Children	2013[2,3]

Environmental	Altitude	Increasing altitude is protective	Adults and children	2007[73], 2013[3]
	Precipitation	Increasing precipitation is protective	Children	2013[3]
	Agriculture	Increasing agricultural land cover increases risk	Children	2013[3]

These various factors highlight the multi-faceted nature of malaria and the many different forces that drive transmission. They also highlight potential inconsistencies in the literature (such as the effect of female sex or community-level wealth).

Interventions in the DRC:

In the DRC, bed-nets and IPTp are two main components of the malaria intervention program[20]. The Ministry of Health began a national ITN and LLIN rollout campaign in 2007[75]. In 2008, the World Bank financed a program to distribute ITNs to Kinshasa specifically[75]. Reported ITN and LLIN ownership increased between 2007 and 2013 from 28% to 72% and 9% to 70%, respectively[2,76]. Additionally, IPTp using SP is recommended for all pregnant women, though estimates from 2013 indicated that only 14% of pregnant women reported receiving at least two doses of SP (three doses are recommended)[20,26,77]. These programs are supported by groups such as The World Bank as well as The President's Malaria Initiative and Santé Rural, two health organizations working in the DRC[20,78]. As the burden of malaria remains high within the DRC, the actual protective effects of these intervention programs need to be investigated.

There is no current national IRS program in the DRC. Limited IRS programs are conducted by a few privately-owned mining companies located in the southern provinces; however, these are very localized programs[79]. In 2017, approximately 36,000 households were included in an IRS program carried out in Luabala province[80]. Future malaria control programs may include IRS, though this would require extensive entomological monitoring[80].

Several studies from the DRC have demonstrated that community-level interventions are associated with reduced malaria risk more so than individual interventions. This has most clearly been demonstrated in the case of bed-nets, where increasing community bed-net coverage was associated with a larger individual protective effect than individual bed-net use amongst children[2,3]. A 2014 study of all-cause child mortality (ACCM) in seventeen African countries (including the DRC) found that the protective effects of individual ITN ownership depended on community-level ITN coverage; individual ITN ownership was only associated with decreased hazard of ACCM in areas with >30% community ITN use[81]. The same study found that community ITN coverage above 50% was protective against malaria amongst children without ITNs within their households, though this specific analysis did not include the DRC[81]. The finding that one person's protection helps protect others is not surprising, and has in fact been a part of intervention planning for many infectious diseases for many years. However, the extent to which community-level coverage of other interventions, such as IPTp, affect malaria risk amongst adults in the DRC has not been explored.

Overview of spatial modeling:

Often, health data are spatially correlated, meaning that disease outcomes within certain geographic areas are likely to be similar[82]. In many cases, individuals who live close to each other have outcomes more similar to each other than those who live farther away. This is not surprising since people who live near each other are exposed to the same environmental factors, interact with each other such that they spread infectious diseases, or are similar demographically. Understanding this spatial dependence is not only valuable in and of itself, but it is also critical for properly estimating any exposure/outcome associations that exist within the space. The fundamental assumption of generalized linear models is that of independent observations;

spatially correlated observations violate this assumption. Luckily, the disciplines of geostatistics and spatial epidemiology specifically work to address this problem.

There are several approaches that have been developed to properly model spatial data and the literature on these topics is vast. A discussion of these methods could (and does) span multiple textbooks, so here we will only briefly discuss the methods we used in our analyses. In this dissertation, I primarily utilized Conditional Auto Regressive (CAR) modeling. CAR models are useful in a setting in which data are aggregated to a set of non-overlapping areal units such as states, counties, or districts[83]. Data from areas that are neighbors are assumed to be more highly correlated than those from areas that are not. Multiple groups have proposed various specifications of this type of model and specifics regarding the models used in this dissertation are provided in Chapter Four. Additional modeling methods that we explored, though ultimately did not include in the analysis, are Spatially Varying Coefficients (SVC) modeling. First laid out by Gelfand et al in 2003, SVC methods allow exposure/outcome associations to vary over space[82,84]. This not only more accurately estimates associations than non-spatial models, but also allows us to discern patterns of effects across space. For example, Wheeler et al demonstrated that the association between the number of alcohol outlets within a neighborhood and the violent crime rate changed considerably across the study area (the city of Houston), with increasing alcohol outlets displaying a protective effect in some neighborhoods but a harmful effect in others[82]. These methods are necessary to properly assess and address spatial autocorrelation, and the choice of proper methods is determined by the research question and available data.

Innovation:

This dissertation is innovative in several ways. First, we combined multiple forms of data: demographic data from the DHS, genetic drug resistance data, and geospatial data, bridging

the fields of epidemiology, genetics, and spatial statistics. Additionally, this study is the first to use nationally representative PCR data to study changes in malaria prevalence in the DRC. As PCR is expensive to conduct, and requires a laboratory and trained technicians, it is not commonly used as a diagnostic for malaria. However, it is the most sensitive diagnostic tool available[9]. Additionally, this is the first study to compare changes in malaria prevalence over time amongst adults in the DRC. It is also the first study, to our knowledge, to evaluate the association between intervention use and changes in malaria prevalence amongst adults in the DRC.

CHAPTER THREE: SPATIAL AND EPIDEMIOLOGICAL DRIVERS OF P. FALCIPARUM MALARIA AMONG ADULTS IN THE DEMOCRATIC REPUBLIC OF THE CONGO

Introduction:

Malaria remains an important cause of morbidity and mortality in the Democratic Republic of the Congo (DRC), which is home to 12% of all cases globally[85]. Understanding malaria transmission in the DRC is critical for furthering efforts to eliminate malaria in sub-Saharan Africa. In addition to the high disease burden, there is evidence that the DRC acts as a bridge of transmission, connecting parasites from East and West Africa[72,73,86]. To combat transmission and reduce disease burden, it is important to determine risk factors for infection. These factors can be used to identify individuals or groups more likely to become infected and, therefore, more likely to benefit from programmatic interventions. Adult infections remain understudied, as most malaria deaths occur amongst children[87,88]. However, adults are frequently infected, often asymptomatically, and may serve as a reservoir for transmission[68,69]. Yet, we know very little about risk factors for infection in adults. In this study, we assessed malaria risk factors amongst adults using data from over 16,000 participants from the nationally representative 2013-2014 Demographic and Health Survey (DHS) conducted in the DRC, the largest and most recent health survey conducted in the country.

A small number of studies performed in the DRC have identified important risk factors for infection[2,3,73]. These studies showed that increasing age, wealth, and individual bed-net use are protective, as are increasing community-level bed-net use, lower average temperature and higher altitude[2,3,74,89]. However, these studies included only children under the age of five[2,3], or

were small and geographically limited[74,89]. A study of adults in the DRC conducted using data from the 2007 DHS identified several risk factors, such as younger age, male sex, and lower individual and community-level wealth[5]; however, no nationally representative risk factor studies of adults have been conducted since.

In this study we evaluated current individual, household, community, and environmental risk factors for malaria amongst adults. Additionally, because increasing antimalarial drug resistance is a growing concern in the DRC, we sought to understand the relationship between community-level resistance and infection prevalence[39,53]. Similarly, the association between increasing antimalarial use and malaria prevalence amongst adults has not been studied in the DRC. Understanding the role of these factors is critical for determining drivers of malaria infection. The different scales of the factors demonstrate the various levels at which malaria interventions can be targeted.

This study builds on previous work in the DRC by evaluating the role of urbanization on risk factors for infection. Understanding the effect of urbanization on malaria transmission is a critical part of intervention planning[4,57,58]. Unlike other infectious diseases, which thrive in cities due to increased population density, malaria transmission is often lower in urban areas as compared to rural areas[59,60]. This is due to several factors, including reduced vector populations, lower biting rates, and better access to therapeutics[59]. As a result, the effects of malaria control programs have been shown to differ between cities and rural areas. Multiple studies conducted in Nigeria and Benin found that bed-net ownership and use were higher amongst rural populations than urban[90–92]. Past work conducted in Liberia determined that the effects of bed-nets differed between the settings, with increasing community-level bed-net coverage being protective in urban areas but displaying no effect in rural areas[63]. We explored this relationship in the DRC by

assessing whether the effects of various risk factors for infection differed between individuals in urban versus rural areas.

The findings from this study can be used to identify individuals and communities at higher risk for malaria infection in the DRC. They also shed light on differences in the epidemiology of malaria between urban and rural settings.

Methods:

Study Population: The data for these studies were drawn from both the 2007 and the 2013-2014 Demographic Health Surveys (DHS) conducted in the Democratic Republic of Congo. The DHS Program, run by USAID in conjunction with local governments, carries out cross-sectional surveys in over 90 countries[93]. In the DRC, the DHS is conducted by several governmental ministries, using a nationally representative randomized cluster sampling method[76]. First, sampling cluster sites were selected from a map of enumerated areas across the country with the probability of selection proportional to the size (number of households) of the cluster. Next, households were randomly selected for inclusion from within clusters[94]. Cluster sizes differ between urban and rural areas with approximately 5 – 15 more households included in rural areas[94]. To account for this nested cluster sampling strategy when analyzing the data, each cluster was assigned a sampling weight. DHS survey conductors visited selected households, obtained consent, and administered an extensive questionnaire covering a broad range of topics including nutrition, education, health history, and infectious diseases[76]. Geolocation data were also recorded for each cluster and randomly jittered between 2 and 10 kilometers. Blood spots were saved on filter paper and shipped to The University of North Carolina for PCR diagnostic testing.

A total of 18,257 adults from 12,549 households were included in the 2013-2014 survey and asked to provide blood samples. This included 9,601 women (ages 15-49) and 8,656 men (ages 15-59). In addition, 9,790 children (ages 6 months to 5 years) were part of the survey. Although they were not included in this analysis, they have been examined previously[2,3,9].

Risk factor selection and modeling: Potential risk factors *P. falciparum* infection were determined by consulting previous studies and based on biological plausibility. We selected individual, household, and cluster (community) level risk factors that have demonstrated associations with malaria risk [2,3,5]. Individual factors included age, biological sex, HIV infection status, education, and wealth index. The DHS Program calculates wealth index as a composite variable based on each household's assets and housing materials[95]. Using Principal Components Analysis (PCA), participants were then grouped into wealth quintiles: poorest, poor, middle, rich, and richest[95]. We also assessed bed-net use, which was determined by whether the individual reported sleeping under a long lasting insecticide net (LLIN) the previous night. At the household level, we constructed a "net ratio" variable by dividing the number of total nets per household by the number of individuals within the household. This was then dichotomized into ratios of less than 0.5 versus those of 0.5 or higher (i.e.: at least one net for every two household members). We also created a composite "housing materials" variable, dichotomized as either traditional or modern, based on data from the roof, wall, and floor material variables (further details are available in Appendix A)[96]. A recent study from The Gambia demonstrated that metal roofs were protective against malaria infection[97], thus we also assessed the relationship between malaria and metal roofing alone, separate from modern housing. Cluster-level factors included proportion reporting LLIN use, median wealth index, and median education level. We also assessed average annual precipitation and temperature as well as the

range in temperature (defined as the difference between the average temperature in January and average temperature in July) and the cluster vegetation index[98]. Data were drawn from the DHS questionnaire as well as from the DHS Program, which collects a range of environmental and geological data[98].

PCR diagnosis: The primary outcome for Aim 1 was *P. falciparum* malaria infection as determined by real-time PCR. In this Aim, malaria is defined as infection with *P. falciparum* parasites. PCR was performed on the blood-spots collected as part of the DHS survey. Whole blood collected by finger-prick was spotted onto Whatman filter paper, dried at ambient temperature, and initially stored with desiccant at -80C in Kinshasa until punching (one 6mm punch per subject) and shipment to UNC. DNA was extracted from the blood spots using a Chelex extraction assay and used for PCR testing[99]. The PCR assay detects the *P. falciparum* lactate dehydrogenase gene (*pfldh*) with a limit of detection of 5-10 parasites/mL[100,101]. The human beta-tubulin (HumTuBB) gene was used as a positive control, and any samples that failed to amplify HumTuBB were excluded. The duplexed *pfldh* and HumTuBB quantitative PCR assay was performed using reaction conditions, primers, and quality control measures for high-throughput PCR exactly as previously described[101]. All samples were run in duplicate. Samples that amplified *pfldh* in only a single replicate were considered negative if the cycle threshold value was higher than 38. All laboratory assays were completed at the University of North Carolina at Chapel Hill.

Genetic analyses: Community-level drug resistance was determined using second-generation sequencing data obtained using molecular inversion probes (MIPs) that target known molecular markers of resistance to antimalarial drugs[53]. MIPs are a technology for obtaining highly multiplexed deep sequencing data that have recently been applied to *Plasmodia* species[53].

Using previously generated sequencing data from 1,065 children enrolled in the 2013-14 DRC DHS[39,53], we assessed single nucleotide polymorphisms (SNPs) of the *pfdhps (*A437G, K540E, and A581G) and *pfcrt* (K76T) genes known to be associated with sulfadoxine-pyramethamine (SP) and chloroquine resistance, respectively[53,102–104]. We used these individual-level data to estimate cluster-level prevalence for each SNP using the *PrevMap* package in R, which fits a spatial model using a Gaussian Process[105]. We fit the model to generate estimates of the underlying allele frequency distribution using maximum likelihood and running 10,000 simulations. Further model details are available in Appendix A.

Urban/rural classification: Potential misclassification of urbanicity is a concern in large surveys with complex sampling frames[106]. In order to minimize such misclassification, we conducted a PCA incorporating variables with a demonstrated relationship to urbanicity [49,98,107]. These variables were: degree of built environment, nighttime lights, total population as of 2014, population density as of 2014, and estimated travel time to the nearest city. The DHS collects these data from a variety of remote sensing and modeling databases including: WorldPop, the Joint Research Centre, and the Climate Research Unit at the University of East Anglia [98,108–110]. The DHS collects these variables for each cluster; all variables were scaled and log transformed for the analysis. From the PCA, we extracted the values for the first principal component (PC) for each cluster, and generated a new urban/rural variable by dichotomizing the PC1 value at the 75% percentile (e.g., urbanicity is represented by the upper quartile of PC1). The new measure of urbanicity was used to assess differences in risk factors between urban and rural areas. We compared the classifications generated for this analysis to the urban/rural categorizations from the DHS using Pearson's correlation coefficient.

Modeling and statistical analyses: All analyses were performed using the R statistical platform v. 3.5.2[111]. We assessed the relationship between each risk factor and PCR detectable malaria infection using bivariate log-binomial regression models (i.e. not adjusted for other variables) to estimate prevalence ratios. We fit models incorporating sampling weights using the *survey* package in R, which uses weighted generalized estimating equations to properly account for the multi-level sampling scheme and correlation between individuals in the same sampling cluster[112,113]. This allowed us to assess individual and cluster-level factors simultaneously. As most households had only one adult member, we did not need to account for within-household correlation. We used bivariate models as the aim was to assess marginal associations, not causal relationships[114]. Each identified risk factor was then further assessed to determine if the association with malaria prevalence differed by urban/rural status by including the urban variable and an interaction term between each factor and the urban variable in the model. As done previously for MIP genetic data, we generated spatially smoothed prevalence and standard error estimates using the *PrevMap* package (**Appendix A**)[105].

In addition to the risk factor modeling we conducted several subsequent analyses. As the DHS asks multiple questions regarding bed-net use, we conducted a sensitivity analysis to compare various coding methods for net use. Further details are presented in the Appendix A. We also assessed whether the association between individual net use and malaria risk differed across categories of overall cluster malaria prevalence. For this analysis, we used previously published data from children included in the 2013-2014 DHS to determine community-level prevalence in order to avoid including the outcome data of adult infections from 2013 in determining cluster-level prevalences[2,9].

Results:

Our analysis included 16,126 adults from the original 18,257 initially selected for inclusion in the DHS. Individuals were sampled from 533 geographically dispersed clusters; GPS data were missing for 44 clusters, resulting in 489 clusters for analysis (**Figure 3.1**). Characteristics describing the individuals with missing GPS data are available in Supplemental Table A2. After final data processing, 16,363 individuals had complete PCR and DHS covariate data. Two-hundred and thirty-seven individuals were not considered "du jure" (members of the sampled household, rather than visitors who slept in the household the previous night[115]) and, thus, were not included in the analysis, resulting in a final data set of 16,126 individuals (**Figure 3.2**). Overall PCR prevalence of *P. falciparum* infection was 30.3% (SE = 1.1). The results of the *PrevMap* analysis demonstrated the high spatial heterogeneity of infection; community prevalence estimates ranged from 0-76% (**Figure 3.3**). The map of the model standard errors indicated low variance in the estimates across the DRC (**Supplementary Figure A1**). Prevalence estimates by province ranged from 6.7% in Nord-Kivu to 58.3% in Bas-Uele (**Supplementary Table A1**).

Reported bed-net use was low; though 75% of adults reported owning a LLIN, only 54% reported sleeping under a net the previous night. The average ratio of nets per household member was 0.27, indicating approximately one net per four household members. Only 17% of adults lived in houses with at least one net per two household members; 24% of all households had at least one net for every two people. Baseline characteristics of study participants are presented in **Table 3.1**.

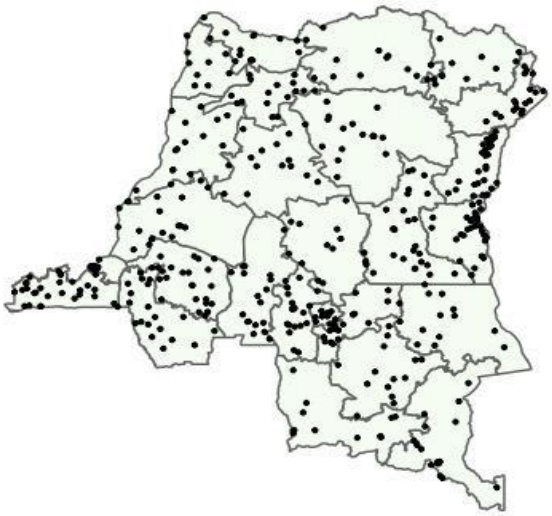


Figure 3.1: Sites of 2013-2014 DHS sampling clusters

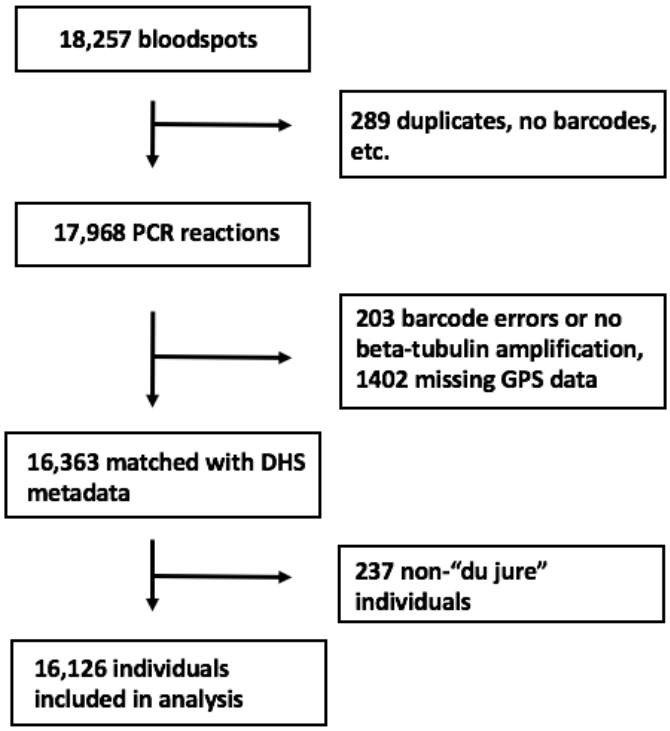


Figure 3.2: Flowchart of samples included in analysis

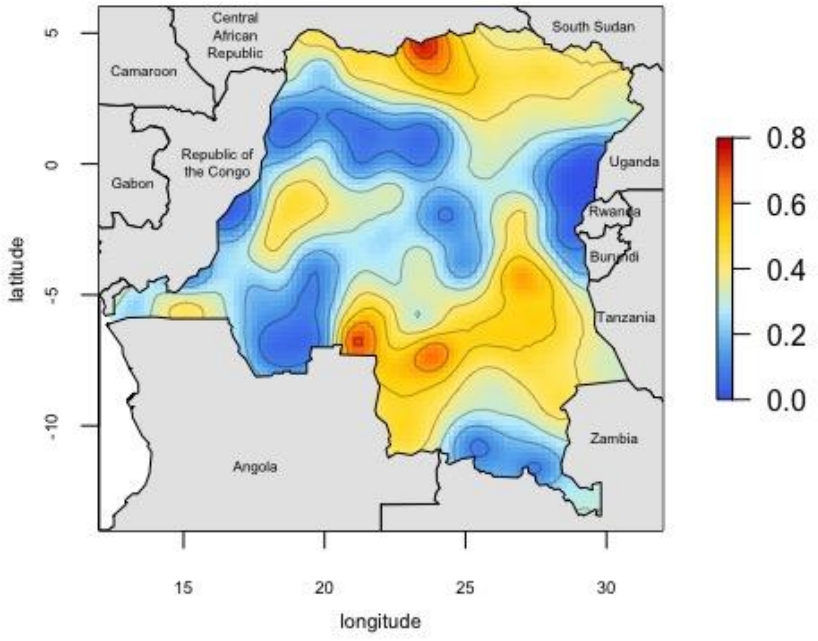


Figure 3.3: Map of predicted *P. falciparum* PCR prevalence estimates**.** Smoothed prevalence estimates, incorporating the sampling weights, were generated using the *PrevMap* package in R[39]. Predicted proportions range from 0-0.76.

Risk factor analysis: Several covariates were associated with prevalence of malaria infection (**Table 3.2**). Individual protective factors included: increasing age, female sex, increasing education, and increasing wealth index. Protective household factors included living in a house made of modern housing materials and having a metal roof. At the cluster-level, increasing median wealth index was protective. Higher use of SP amongst pregnant women at the cluster-level was also found to be associated with lower prevalence of malaria. Drug-resistance mutations were more common in low-prevalence clusters. Increasing average temperature was associated with increased prevalence of infection as was increasing vegetation. A larger range in average monthly temperature was associated with lower malaria prevalence. Full risk factor modeling results are available in Table 3.2.

We found a modest protective association of individual LLIN use and no effect of increasing community LLIN use, in contrast to previous findings in the DRC[2]. At the household

level, a net ratio greater than 0.5 (i.e. at least one net per two household individuals) was protective, compared to households with a lower ratio. When we stratified individual net use by insecticide type, we observed a protective effect of deltamethrin-treated nets and a signal for protection with alphacypermethrin nets. There was no significant protective effect of permethrin-treated nets, a finding that has been previously reported for children in the DRC[2]. Restricting LLIN use to only those who had reported using a net treated with deltamethrin or alphacypermethrin, we found a slight protective effect of increasing community-level net use. The sensitivity analysis of net use showed no differences between measurement options or the coding choice for net use (**Supplemental Figure A1**). Additionally, the sensitivity analysis showed no difference in the association when restricting "net-use" to only nets under 3 years old. A comparison of individual LLIN net use between adults and children included in the 2013-2014 DHS indicated similar protective effects (**Supplementary Table A4**).

Analysis of Associations by Urban/Rural status: There were several socioeconomic-related risk factors whose association varied by urban/rural status (**Figure 3.4**). These included individual-level wealth, with increasing wealth showing a protective effect in urban areas but not rural. A similar trend was observed for increasing individual-level education. Additionally, both modern housing and metal roofs were protective in urban areas but not in rural areas. Cluster-level wealth demonstrated problematically high collinearity with urbanicity and thus could not be modeled. Full numeric results from this analysis are presented in **Supplemental Table A3.**

Overall, urbanicity did not impact the association between LLIN use and malaria prevalence, either at the individual or cluster-level. However, when we subset LLIN use to only deltamethrin and alphacypermethrin nets, the only insecticides that demonstrated a protective effect in the initial analyses, we observed that increasing individual and community use of these

nets were more protective in rural areas than in urban areas. Similarly, the analysis of LLIN use by cluster-level prevalence found that individual net use was more protective in areas of higher overall malaria prevalence (**Figure 3.5**).

The correlation between the urban/rural categories generated for this analysis and the original DHS categories was 0.75.

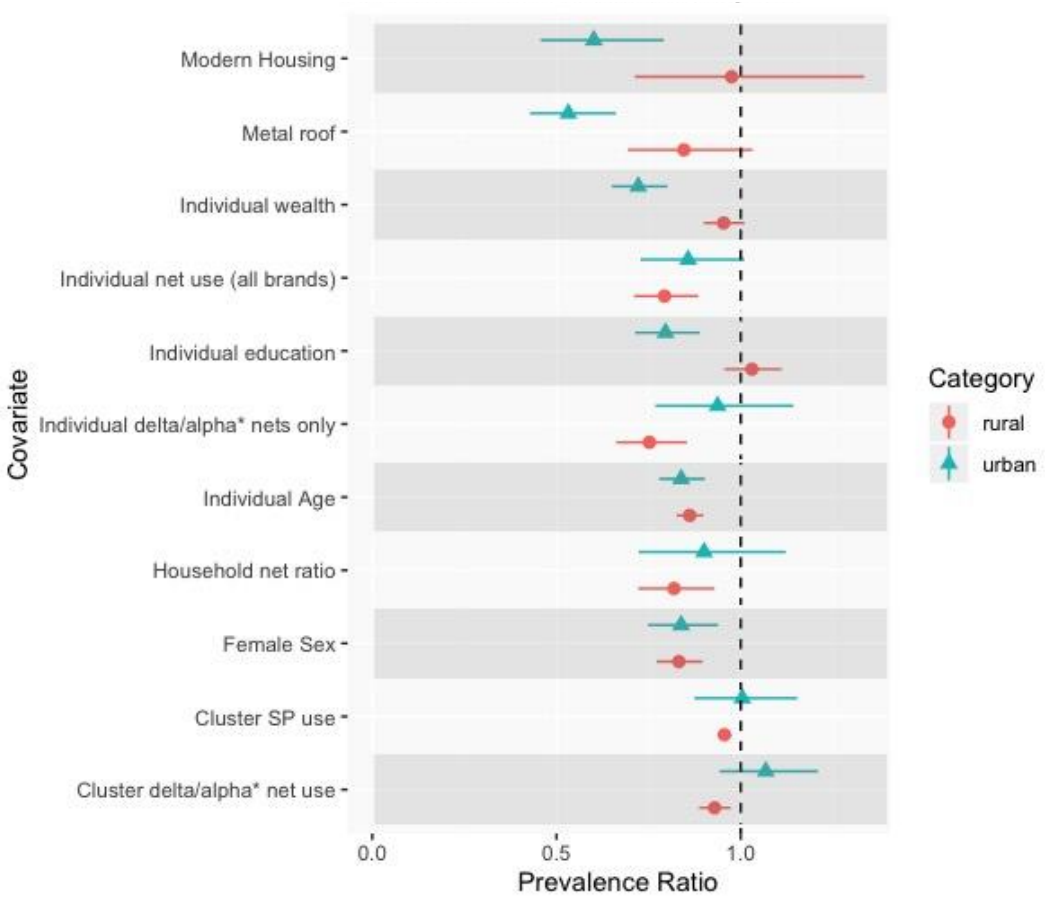


Figure 3.4: Results of the analysis comparing risk factor effects between urban versus rural areas. Prevalence ratios and confidence intervals by urban status are presented for each risk factor. Urban results are presented with blue triangles, rural results with red circles. Differences in point estimates indicate differences of the prevalence ratio by urbanicity. The associations of several factors, such as modern housing, education, and wealth, demonstrated differences between urban and rural areas. The null value (Prevalence Ratio = 1) is indicated with a vertical dashed line.

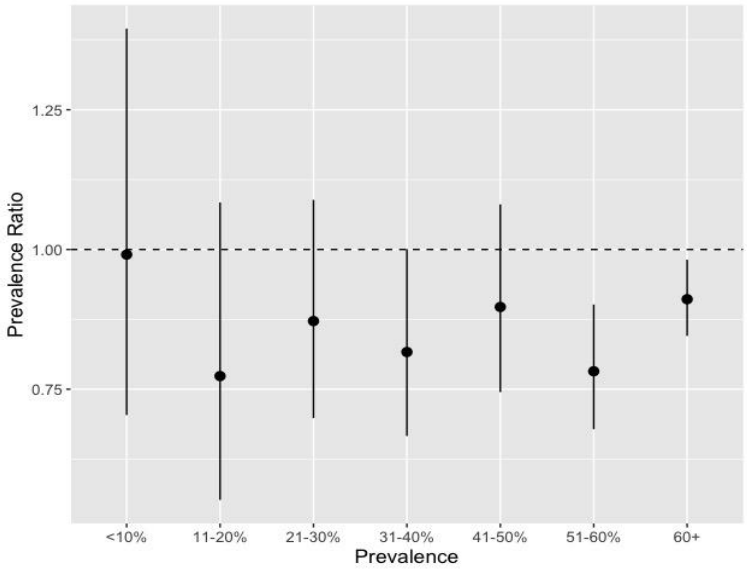


Figure 3.5: Effect of individual bed-net use by cluster-level malaria prevalence. We used previously published data from children included in the 2013-2014 Demographic and Health Survey in order to determine cluster-level prevalence[9]. Individual bed-net use was more protective in clusters with higher malaria prevalence. The null value (Prevalence Ratio = 1) is indicated with a horizontal dashed line.

Discussion:

In this study, utilizing data from the largest and most recent nationally-representative health survey conducted in the DRC, we found a high national prevalence of *P. falciparum* malaria (30%) amongst adults. We also found high spatial heterogeneity. Community-level prevalence estimates reached as high as 76%. Malaria is often considered a pediatric disease as the majority of deaths occur amongst children[87]. However, our findings emphasize that the burden of disease amongst adults is high in the DRC and highlight the importance of adult infections in malaria epidemiology. Addressing adult infections is critical for eliminating malaria as adults may serve as a reservoir for further transmission of parasites[68]. The high level of spatial heterogeneity across the country underscores the need to study malaria on a sub-national scale and develop targeted strategies for areas of highest prevalence (and thus a larger reservoir). The spatial distribution of infections amongst adults matches that of infections amongst children

included in the 2013-2014 DHS, indicating similar underlying spatial processes[116]. Similarly, the prevalence map displays similar a pattern to that from adults included in the 2007 DHS[5]. The areas of high versus low prevalence are likely driven by several factors such as altitude and urbanicity. The mountainous regions of Kivu in the northeast have very little malaria, while the mining districts in southern DRC have higher prevalence.

The national prevalence estimate of 30% amongst adults is similar to that from the 2007 DHS, suggesting a need for increased investment in malaria control in the DRC and further understanding of drivers of transmission[5,85]. We observed that the prevalence is unchanged from 2007 despite increases in LLIN coverage and the number of individuals being tested and treated for infection[85]. Thus, understanding malaria risk factors in all age groups remains critical for the design and implementation of effective interventions. Several risk factors identified in an analysis of the 2007 DHS data were also associated with increased infection prevalence in this study, highlighting their continued importance in the epidemiology of malaria. These include younger age, male sex, and lower community-level wealth[5]. The factors identified in this study, many of which have also been identified in other countries[10,117,118], are particularly important for identifying infected individuals in the DRC, as many adult infections are asymptomatic and often go undetected[68]. Thus, targeting malaria interventions towards younger adults, men, and poorer communities could maximize their impact.

In this study, we found that individuals living in households with a bed-net ratio greater than 0.5, i.e.: at least one net for every two household members, had lower prevalence of malaria. This ratio is recommended by the WHO in order to ensure a sufficient number of nets for household members[14]. Though previous studies have assessed the role of increasing community-level net use, the effect of within household "net coverage" has not been studied, to our

knowledge. Our findings demonstrate the importance the number of nets within a household and support the WHO recommendation. However, only 24% of households in 2013-2014 DHS had a net ratio of 0.5 or higher. The 2019 WHO World Malaria Report estimated that currently, approximately 50% of households in the DRC have at least one net for every two people[11]. Thus, the DRC is making progress towards achieving the WHO recommendation; however, there is still a lot of work to be done to make sure all households meet the recommendation. It is critical that future bed-net distribution campaigns ensure that enough nets are provided to each household.

In this analysis we did not observe the overall protective effect of community bed-net use that has been previously demonstrated amongst children in the DRC, though there was a protective effect of increasing community use of deltamethrin and alphacypermethrin treated nets[2]. As mentioned, we did see a protective effect of increasing within household net coverage, which may be a more important factor for reducing infection than community net use. Our findings of greater protection from nets in higher prevalence areas and in rural areas are supported by a recent meta-analysis that found increasing community malaria prevalence associated with a greater protective effect of LLIN use (OR = 0.80) [119]. Thus, bed-net distribution campaigns may be more successful in rural or generally higher prevalence areas than in cities.

Poor LLIN efficacy may be due to several factors such as adult sleeping behaviors and changing mosquito biting patterns, and demonstrates that malaria control programs cannot rely on LLIN use alone. Many adults go to sleep after *Anopheles* mosquitoes begin biting, rendering LLINs less useful[120]. Additionally, recent studies have demonstrated a shift in *Anopheles* biting hours earlier in the evening as a result of LLIN use[121]. These findings point to the need to

consider additional malaria control programs such as improved community testing for malaria and targeted mass drug administration[122–124].

The findings from the genetic analysis of mutations associated with SP and chloroquine resistance indicate that areas of higher prevalence of drug resistance had lower prevalence of malaria infection. While this is a marginal association and does not reflect a causal relationship, this could be due to the amount of anti-malarial drug use, leading to lower overall prevalence of malaria but increased resistance through increased selective pressure. Future studies should aim to investigate the causal effect of increasing community-level drug resistance on malaria risk; however, this was not the primary aim of this study. Additionally, cluster-level SNP prevalences were modeled estimates generated using data from children and thus may not be representative of overall community-level prevalence.

Modern housing and metal roofing were both associated with lower prevalence of malaria, though the association was more pronounced in urban areas than in rural areas. The effect of metal roofing agrees with findings from a recent study conducted in the Gambia, which found 38% lower mosquito survival and lower malaria prevalence amongst villages with higher proportions of metal roofs[125]. The authors of the study propose that this is due to the higher temperatures of metal roofs during the day, leading to lower mosquito survival. The present findings suggest that housing improvements may help reduce malaria risk, either directly as proposed by the Gambian study, or indirectly through overall improvements in living conditions and socioeconomic status.

This study also highlights differences in the epidemiology of malaria between urban and rural areas, confirming the need to tailor interventions to different populations in cities versus rural areas. Increasing wealth and education are both highly protective in urban areas but not in

rural areas. This may be a result of increased access to prevention and treatment methods in urban areas, as has been observed in other countries. A study from Equatorial Guinea found individuals in rural areas waited longer to seek treatment for malaria infections compared to those in urban areas, and were more likely to be treated at home rather than in a health facility[126]. These results also agree with findings from previous studies that poor individuals living in urban areas have similar health risks to the general rural population[58]. Thus, in urban areas, the malaria positive population is poorer and less educated than those infected in rural areas. Malaria control programs should take these differences in high-risk populations into account, targeting poorer or less educated individuals in cities and ensuring that interventions are accessible for these populations. Conversely, interventions in rural areas need to be more widespread and accessible to a more diverse population.

This study has several strengths. First, it uses nationally-representative, population-based data from over 16,000 individuals, the largest health survey conducted in the DRC. This allows us to make inferences regarding the country as a whole. Other recent malaria risk factor studies have fewer individuals or are conducted in a smaller geographic area[74,89]. Second, we determined malaria infection using high-throughput real-time PCR, the most sensitive method for diagnosing infection[9]. Third, it leverages country-wide drug resistance genotyping data to inform community-level epidemiological analysis. Lastly, this study incorporates multiple types of data including: survey, molecular, deep sequencing drug resistance, and geospatial data collected at the individual, household, community, and environmental level. This allowed us to demonstrate the different scales of malaria risk factors; control programs should aim to intervene at each of these levels.

The findings from this study are subject to limitations. Several of the covariates included in the study were obtained from self-reports, including LLIN usage. While self-reported data are subject to recall bias, the DHS questionnaire asks multiple questions regarding net use that allowed us to assess bias. The sensitivity analysis comparing these questions indicated no difference in modeling results between the questions, providing confidence that bias from self-reports is minimal. Secondly, this analysis evaluated marginal associations and thus the findings cannot be interpreted as causal effects. However, the associations are useful for identifying and targeting interventions to higher risk individuals and groups such as younger, less educated men. Additionally, as the DHS is a cross-sectional survey, we did not assess the effects of seasonality. Finally, we could not directly assess the epidemiology of symptomatic malaria in the DRC as DHS surveys do not sample health facilities.

Conclusion:

This study evaluated risk factors for *P. falciparum* infection amongst adults in the DRC using data from the nationally representative, population based DHS survey. Overall prevalence of infection was high, 30%, and grossly unchanged from the prior 2007 survey. We observed high spatial heterogeneity across the country and identified individual, household, and community-level risk factors for malaria such as male sex, traditional housing, and decreased within household net coverage. These findings support the need for sustained investment in malaria control in the DRC and can be used to develop targeted interventions with maximal impact.

Table 3.1: Descriptive statistics of the study population by *P. falciparum* PCR status

	PCR-positive	PCR-negative	Total
Unweighted total number*	5,372	10,754	16,126
Weighted Proportion**	30.3%	69.7%	
***Individual Level**:**			
Median Age (IQR)	26 (19-36)	29 (21 - 38)	28 (29-38)
Number Female (%)	2,360 (47.6)	6,202 (54.4)	8,562 (52.3)
Number HIV positive (%)	30 (0.6)	126 (1.1)	156 (0.9)
Education Category (%)			
No school	515 (10.4)	1,140 (10.0)	1,655 (10.1)
Primary school	1,618 (32.6)	3,346 (29.4)	4,964 (30.4)
Secondary school	2,662 (53.8)	6,079 (53.4)	8,741 (53.5)
Higher than secondary	156 (3.2)	826 (7.2)	982 (6.0)
Owns a bed-net (%)	3,615 (72.9)	8,589 (75.3)	12,204 (74.6)
Slept under bed-net previous night (%)	2,462 (49.7)	6,422 (56.3)	8,884 (54.3)
Wealth Category (%)			
Poorest	1,059 (21.4)	1,840 (16.1)	2,899 (17.7)
Poor	1,030 (20.8)	2,014 (17.7)	3,044 (18.6)
Middle	1,122 (22.6)	2,124 (18.6)	3,246 (19.8)
Rich	1,041 (21.0)	2,249 (19.7)	3,290 (20.1)
Richest	704 (14.2)	3,178 (27.9)	3,882 (23.7)
Household Level:			
Average number of bed-nets per person (SE)	0.25 (0.07)	0.27 (0.08)	0.26 (0.07)
Modern Housing (%)	593 (12.0)	2,514 (22.1)	3,107 (19.0)
Metal Roofing (%)	1,564 (31.6)	5,147 (45.1)	6,711 (41.0)

Cluster-level**:

Median Age (IQR)	30.0 (28.5 – 31.3)	29.7 (28.2 – 31.0)	30.0 (28.6-31.6)
Urban (%)	1,329 (26.8)	4,297 (37.7)	5,626 (34.4)
Median Education (IQR)	2 (1-2)	2 (1-2)	3 (2-3)
Median Wealth Score (IQR)	3 (2-4)	3 (2-5)	3 (2-4)
Average Annual Centimeters of Precipitation (SE)	152.2 (1.4)	149.9 (1.7)	150.6 (1.4)
Average Temperature (SE)	24.7 (0.1)	23.7 (0.2)	24.0 (0.2)
Vegetation Index***	3934.5 (52.6)	3660.7 (69.5)	3734.6 (58.7)
% Drug Resistance****:			
Any *pfdhps*	92.2 (0.7)	95.1 (0.4)	94.2 (0.5)
pfdhps K540E	23.5 (1.7)	32.9 (2.5)	30.1 (2.1)
pfdhps A581G	1.8 (0.1)	3.9 (0.4)	3.2 (0.3)
pfcrt K76T	56.0 (2.3)	64.8 (1.9)	62.1 (1.8)
% Net Ownership (SE)	73.7 (1.3)	75.0 (1.5)	74.6 (1.3)
% Net Usage (SE)	52.7 (1.4)	54.6 (1.6)	54.0 (1.4)
% SP use amongst pregnant women (SE)	25.3 (1.3)	28.0 (1.1)	27.2 (1.1)

*These are unweighted raw numbers and do not represent the sum of the subsequent values in the table as subsequent values incorporate sample weights
**Proportions and numbers with sampling weights applied
***vegetation index ranges from 0 (least vegetation) to 10,000 (most vegetation)[98]
****estimates generated using previously published data[53,127]

Table 3.2: Risk factor analysis results

Variable	Prevalence Ratio	95% Confidence Interval	p-value[†]
Individual Level:			
Age (scaled)	0.86	0.83 – 0.89	**<0.001**
Female Sex	0.83	0.78 –0.88	**<0.001**
HIV positive	0.63	0.38 - 1.03	0.067
Education Category:			
No school (REF)	--	--	--
Primary school	1.05	0.92 – 1.18	0.473
Secondary school	0.98	0.85 – 1.13	0.761
Higher than secondary	0.51	0.38 – 0.70	**<0.001**
Wealth Category:			
Poorest (REF)	--	--	--
Poor	0.93	0.83 – 1.04	0.189
Middle	0.95	0.85 – 1.06	0.330
Rich	0.87	0.73 – 1.02	0.088
Richest	0.50	0.40 – 0.61	**<0.001**
Owns a Net	0.92	0.82 – 1.03	0.138
Slept under LLIN	0.83	0.76 – 0.91	**<0.001**
Net type:			
No net (REF)	--	--	--
Permethrin	0.94	0.78 – 1.12	0.477
Alphacypermethrin	0.68	0.38 – 1.22	0.198
Deltamethrin	0.81	0.73 – 0.91	**<0.001**
Household level:			
Net ratio*	0.85	0.76 - 0.95	**0.003**
Modern Housing	0.58	0.49 – 0.69	**<0.001**

Metal Roofing	0.66	0.58 - 0.75	**<0.001**
Cluster Level:			
Urban	0.70	0.37 - 0.83	**<0.001**
LLIN ownership**	0.97	0.93 – 1.00	0.058
LLIN usage**	0.98	0.92 – 1.04	0.457
LLIN usage (deltamethrin and alphacypermethrin nets only)**	0.95	0.90 - 1.00	**0.040**
Education	0.91	0.79 – 1.04	0.174
Wealth	0.87	0.83 – 0.92	**<0.001**
Precipitation (scaled)	1.06	0.97 – 1.17	0.179
Temperature (scaled)	1.32	1.23 - 1.42	**<0.001**
Temperature range (scaled)	0.91	0.85 – 0.98	**<0.001**
Vegetation Index (scaled)	1.18	1.09 – 1.27	**<0.001**
SP[††] use amongst pregnant women**	0.92	0.89 - 0.96	**<0.001**
Drug Resistance Prevalence**			
Pfdhps A437G	0.88	0.85 – 0.91	**<0.001**
pfdhps K540E	0.95	0.93 – 0.98	**<0.001**
pfdhps A581G	0.89	0.85 – 0.94	**<0.001**
pfcrt K76T	0.93	0.91 – 0.96	**<0.001**

[†] P- value of the test of the null hypothesis that the PR = 1
[††] Sulfadoxine/pyrimethamine
* Ratio >=0.5 vs <0.5
** logit transformed

CHAPTER FOUR: INCREASED PATENT MALARIA IN THE DRC: GEOGRAPHIC AND EPIDEMIOLOGICAL CHANGES FROM 2007 TO 2013-2014

Introduction:

As worldwide efforts to reduce malaria transmission have stalled, there is an increasing need to evaluate the effects of malaria control programs[87]. Despite increased investment in malaria control, there are a number of growing threats to progress. These include increasing insecticide resistance to pyrethroids, an insecticide used to treat many long-lasting insecticide treated nets (LLINs),[128] and resistance to many commonly used anti-malaria therapeutics[53,103,127]. Specifically, mutations of the *Plasmodium falciparum dhps* gene are associated with failure of sulfadoxine-pyrmethamine (SP); the drug provided to pregnant women to prevent infection during pregnancy[4,6]. Considering these threats, monitoring the effectiveness of interventions is critical for ensuring the success of malaria control programs.

Understanding the effects of intervention programs is particularly important in the Democratic Republic of the Congo (DRC), which has the second highest malaria burden in the world[87]. With an estimated 25 million cases of malaria in 2017, the DRC is home to 12% of cases worldwide[11,87]. Additionally, multiple studies have demonstrated that parasites from the DRC are genetically related to parasites from both East and West Africa, indicating that the DRC acts as a bridge of transmission across the continent[72,86]. These findings, along with the large size and population of the country, highlight the particular importance of reducing transmission in the DRC.

There is evidence that the prevalence of malaria in the DRC has remained steady or even increased over the past two decades. The World Health Organization (WHO) estimated that there

were approximately 23,620,000 total cases in 2006 and 23,378,784 in 2013, demonstrating that prevalence remained relatively steady between these two years[11,129]. However, recent WHO estimates also reported an increase of more than 500,000 infections in the DRC between 2016 and 2017[14]. Additionally, the proportion of hospitalizations due to malaria increased from 46% in the 2005 – 2007 period to 55% in the 2011 – 2014 period[20]. Similarly, a recent study found that the number of reported malaria cases increased between 2005 and 2014. However, the authors hypothesize that this is due to an increase in detection and improved diagnostics rather than an increase in transmission[130]. These findings indicate that the state of malaria over the past decade remains unclear. In this study, we aimed to assess changes in malaria prevalence amongst adults using the most recently collected nationally representative, population-based data.

Over the past two decades, the DRC increased investment in malaria control programs. Two main intervention programs are LLINs and intermittent preventive therapy for pregnant women (IPTp)[20]. Indoor Residual Spraying (IRS) is limited and only performed in select areas by private companies; there is no national IRS program[20]. Both the LLIN and IPTp programs have been scaled up through efforts from the Ministry of Health (MoH) and multiple health organizations working in specific regions of the DRC[131]. The MoH and the World Bank began extensive bed-net distribution campaigns in 2007 and 2008[75]. The President's Malaria Initiative (PMI), which supports LLIN distributions and provides SP for IPTp programs, increased its annual budget for control programs in the DRC from approximately 7 million dollars in 2007 to 37 million in 2010[131]. During this period PMI programs also included community-based malaria education and training for health care workers regarding IPTp delivery[131]. However, as the burden of malaria remains high within the DRC, the actual protective effects of these intervention programs need to be investigated.

In this study, we aimed to understand differences in the spatial distribution of malaria between 2007 and 2013 and determine the impact of the LLIN and IPTp programs. As malaria is known to demonstrate high spatial heterogeneity, we sought to understand changes in prevalence on a sub-national scale[3,16]. Evaluating the effects of the intervention programs will help the MoH plan future intervention programs and ensure that control efforts are maximally effective.

Methods:

Study populations: This analysis utilized data drawn from the 2007 and 2013-2014 Demographic Health Surveys (DHS). A full description of the DHS is provided in Chapter Three.

Intervention and covariate data: Data regarding LLIN and IPTp use were drawn from the DHS. The DHS asks several questions regarding LLIN use; for this analysis, we determined LLIN use by whether the individual reported sleeping under an LLIN the previous evening. A previous sensitivity analysis discussed in Chapter Three demonstrated no difference between the choice of LLIN use measurement between the different questions[132]. The DHS also collected data regarding the LLIN brand, which we used to determine the insecticide with which the net was treated. Questions regarding IPTp use asked women whether they received SP during their most recent pregnancy and if so, how many doses. The DHS questionnaire asks all women if they are pregnant at the time of the survey as well as whether they have been pregnant in the previous five years.

Data regarding additional covariates, such as age, wealth index, and level of education, were also drawn from the DHS. The DHS constructs a five-category composite wealth index based on a questionnaire of individual and household assets[95]. Additionally, we assessed anti-malarial use amongst children under the age of 5 (the DHS does not collect these data amongst

all adults). We also included an urbanicity score, a composite variable based on multiple factors with a demonstrated relationship to urbanicity. The method for constructing this variable has been previously described in Chapter Three[132].

Molecular Diagnosis of Patent Infections: The outcome for this study was patent malaria infection, defined as at least 100 parasites per microliter of blood. In both 2007 and 2013, we used quantitative Polymerase Chain Reaction (qPCR) to determine malaria infection. However, the qPCR assays used to detect the presence of malaria parasites differed between surveys, making a direct PCR comparison difficult. The 2007 assay targeted the 18S region of *Plasmodia* ribosomal DNA[133], while the 2013 assay targeted the *P.f.* lactase dehydrogenase gene (*pfldh*), as previously described[101]. For the 2007 survey, DNA was extracted from blood spots using a QIAamp 96 DNA blood kit (Quiagen, Germantown Maryland); DNA was extracted from blood spots from the 2013 survey using Chelex extraction. All samples were run in duplicate.

To determine the most appropriate method of comparison, we evaluated assay performance by running both assays on a subset of 160 samples from the 2013 DHS. Using the 18S assay (used in 2007) as the gold standard, the sensitivity of the *pfldh* assay (used in 2013) was only 57%, though the specificity was 100% (**Supplementary Table B1).** The limit of detection (LOD) also differed between the assays (LOD of the 18S assay = <1 parasite/uL[134]; LOD of the *pfldh* assay = 10-50 parasites/uL[9]). We selected a threshold of 100 parasites/uL in order to directly compare the assays. This cutoff is also the LOD for microscopy, a commonly used method for diagnosing malaria. Thus, this is a clinically meaningful comparison. After applying the 100 parasites/uL thresholds, agreement between the assays was 97.5% (**Supplementary Table B2**). We determined sample parasite densities using standard curves from controls with known densities.

Spatial analysis: The sampling clusters used in the 2007 DHS changed for the 2013 DHS. In 2007, 300 clusters were selected, while a set of 536 new clusters were selected in 2013 (**Figure 4.1**). These changes prohibit a direct comparison of cluster-level malaria prevalence across time. To address this issue, we aggregated the cluster-level data to the 26 provinces of the DRC (**Figure 4.1**), incorporating the survey sampling weights.

Within the boundaries of the 26 provinces, the DRC is further divided into 150 territories (**Figure 4.2**). To assess changes in prevalence on a smaller spatial scale, we also aggregated the data to the territory level. There were seven territories that did not have a DHS sampling cluster in 2013, and 36 with no sampling clusters in 2007. We imputed missing covariate data using ordinary kriging with the *sp* package in R[135]. We did not impute outcome data for the seven territories without prevalence data from 2013; these territories were not included in the models.

We assessed the extent of spatial autocorrelation of the data at the province and territory-level by estimating Moran's I statistics. Moran's I measures correlation in data over distance, ranging from -1 (perfect dispersion) to +1 (perfect correlation)[136,137]. We estimated Moran's I using the *sp* package in R[135]. All statistical tests were performed using the R platform v. 3.6[111].

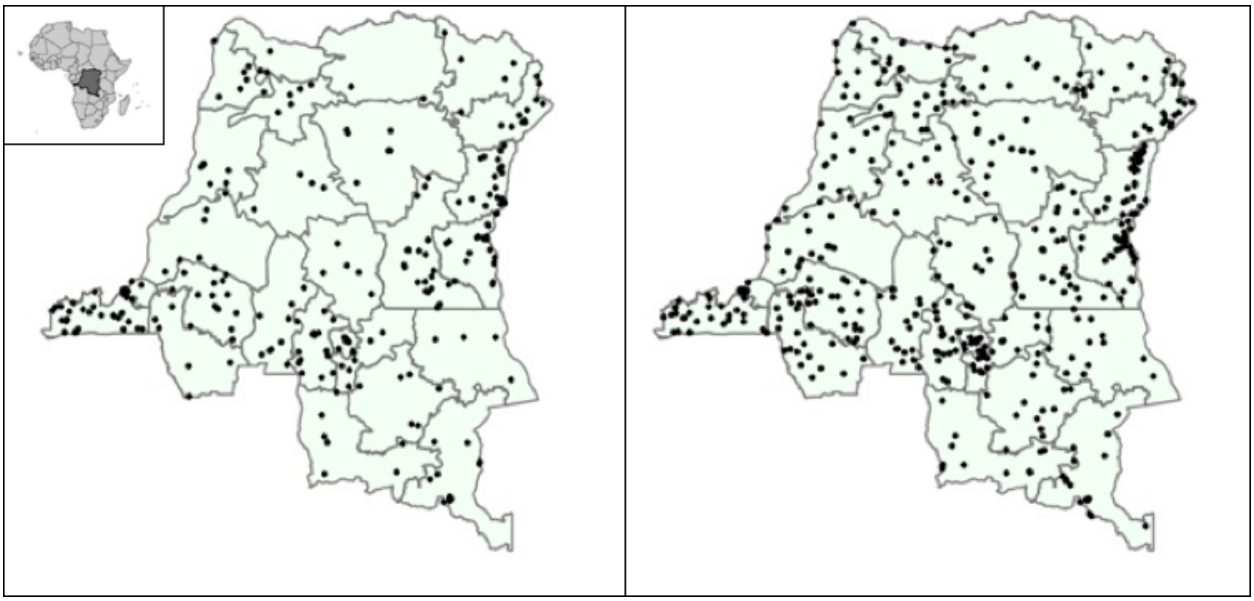


Figure 4.1: Sampling locations from the 2007 (left) and 2013-2014 (right) Demographic and Health Surveys. A total of 300 sampling clusters were included in 2007; 536 clusters were included in 2013-2014. Province boundaries are indicated in black.

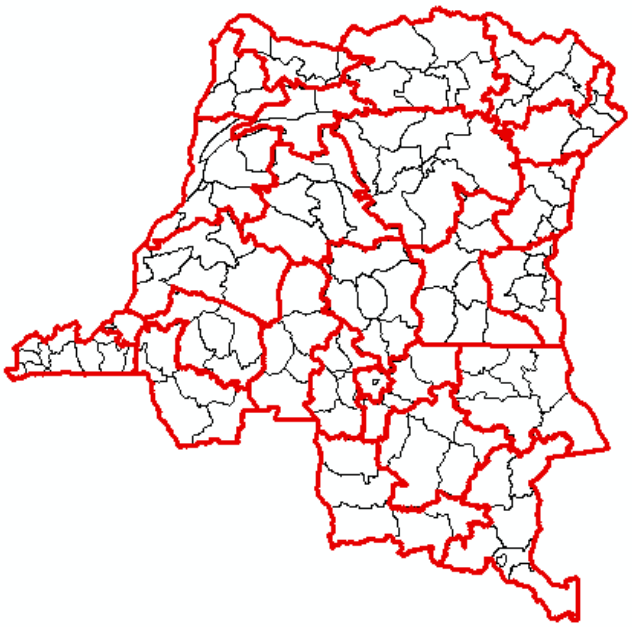


Figure 4.2: Map of the territories within the DRC. The 150 territories of the DRC are outlined in black; the 26 provinces are outlined in red.

Analysis of IPTp:

To assess overall uptake of IPTp, we evaluated IPTp use amongst women who reported a pregnancy in the previous five years. Women were categorized as having received any IPTp, at least two doses of IPTp, or not receiving IPTp. To assess the relationship between IPTp use and patent infections, we evaluated only women who were pregnant at the time of the DHS survey (when the malaria testing was conducted). We assessed the proportion of pregnant women with patent infections who received IPTp versus those who did not receive IPTp using chi-squared tests. We also used Fisher's exact tests if any category had fewer than five women.

LLIN Modeling: As we aggregated data to the territory-level, we chose to use area-level models. These models assessed the relationship between territory-level LLIN use and the change in patent infection prevalence between 2007 and 2013. We fit both standard non-spatial multilevel models including a random effect for province, and models with a spatial random effect in order to incorporate the data's spatial dependence. We compared model fit using the Deviance Information Criterion (DIC) and selected the final models based on the lowest DIC.

We fit models with a spatial random effect using the CARBayes package in R[83]. The models had the following form:

$$Y_k = X^T_k \boldsymbol{\beta} + \phi_k$$

Here, Y_k represents the difference in prevalence for each spatial unit k (each territory), X represents a matrix of covariates for each k and $\boldsymbol{\beta}$ is a vector of regression coefficients linking the covariates to the response variable. Lastly, ϕ represents the spatial random effect. As these were area-level models, we chose a conditional autoregressive correlation structure for ϕ (further

details provided in the Appendix B). The model was fit using Markov chain Monte Carlo and run with 100,000 iterations. Details regarding model priors are provided in Appendix B.

For both sets of models, the primary exposure was the proportion of individuals reporting LLIN use. We assessed the effect of all LLIN use, and use of nets treated with deltamethrin or alphacypermethrin only. We subset to these insecticides as they were the only insecticides that demonstrated efficacy in previous studies[2]. The outcome from the models was the difference in territory-level prevalence between 2013 and 2007. We also fit both crude and adjusted models in order to account for confounders of the effect of LLIN use on malaria prevalence. All proportions were logit transformed.

As a secondary analysis, we also estimated the effect of individual LLIN use and assessed differences in the effects between provinces. To accomplish this, we fit non-spatial multilevel models. These models assumed a binomial distribution with a random intercept for province and a random slope for LLIN use. This allowed the effect of LLIN use to vary between provinces. However, these models did not account for the data's spatial dependence. We fit the models using the lme4 package in R[138] and determined standard errors for random effects using the merTools package[139]. The full model specification is presented in Appendix B.

We determined confounders of the LLIN association with malaria infection based on the literature, biological plausibility, and constructing Directed Acyclic Graphs. Potential confounders of the LLIN-malaria relationship were: age, wealth index, education level, degree of urbanicity, community-level net use in 2007, and patent infection prevalence in 2007. As detailed above, data regarding these factors were drawn from the DHS.

Results:

A total of 8,698 individuals from 2007 and 16,126 from 2013 had available molecular and DHS survey data (**Table 4.1**). In the 2013 DHS, 44 clusters were missing GPS coordinate data and were excluded from the analysis. Individuals who were not considered "du jure" (members of the household rather than visitors) were also excluded.

The national prevalence of patent *P.f.* malaria increased from 2.4% (SE = 0.3) in 2007 to 7.5% (SE = 0.4) in 2013. Province-level prevalence estimates ranged from 0 - 6.6% in 2007 to 1.2 – 15.1% in 2013 (**Figure 4.3**). Full province-level data are available in Supplementary Table B3. The median age of those infected was unchanged between years, 21.5 in 2007 (IQR= 18-31) and 22 in 2013 (IQR = 18-31) (**Supplementary Figure B1**). Use of both LLINs and IPTp increased between surveys, though uptake was heterogeneous across the DRC (**Figure 4.4**). However, clusters with high LLIN use (>50%) also reported higher IPTp use than clusters with lower LLIN use (28% vs 22%, $X^2 = 5.776$, p = 0.016). Overall use of anti-malarial drugs amongst children who had a fever remained steady between 2007 (29.7%) and 2013 (28.2%) (**Supplemental Table B4**). However, the types of anti-malarial drugs shifted between 2007 and 2013, with a higher proportion of children taking Artemisinin combination therapies (ACTs) and quinine in 2013 as compared to 2007 (**Supplemental Table B4**).

Tests of spatial autocorrelation: The results of the Moran's I tests indicated low evidence of spatial autocorrelation at the province-level (I = 0.066, p = 0.175). At the territory-level, the test indicated moderate spatial autocorrelation (I = 0.107, p = 0.019).

Analysis of IPTp results: Overall, the proportion of women who received IPTp during their most recent pregnancy increased between surveys (**Table 4.1**). However, uptake of IPTp

remains low; only 13.6% of women pregnant in the past five years received at least two doses of SP (three doses is recommended) (**Table 4.1**).

Five-hundred and twenty women (12.2%) included in the 2007 DHS were pregnant; 1062 women (12.7%) were pregnant in 2013. Prevalence of patent infections amongst pregnant women increased from 4.2% (SE = 0.8) to 12.3% (SE = 1.0) ($X^2 = 25.326$, $p < 0.001$). Amongst women who received any IPTp, the proportion of infected women was slightly lower than amongst women who did not receive IPTp, in both 2007 [2.1% vs. 4.5%] and 2013 [9.5% vs. 13.1%]. (**Table 4.2**). The proportions of patent infections amongst women who received at least two doses of IPTp was slightly lower than amongst women who did not receive at least two doses in 2007 [0% vs. 4.4%], though the proportions were similar in 2013 [12.1% vs. 12.3%] (**Table 4.2**).

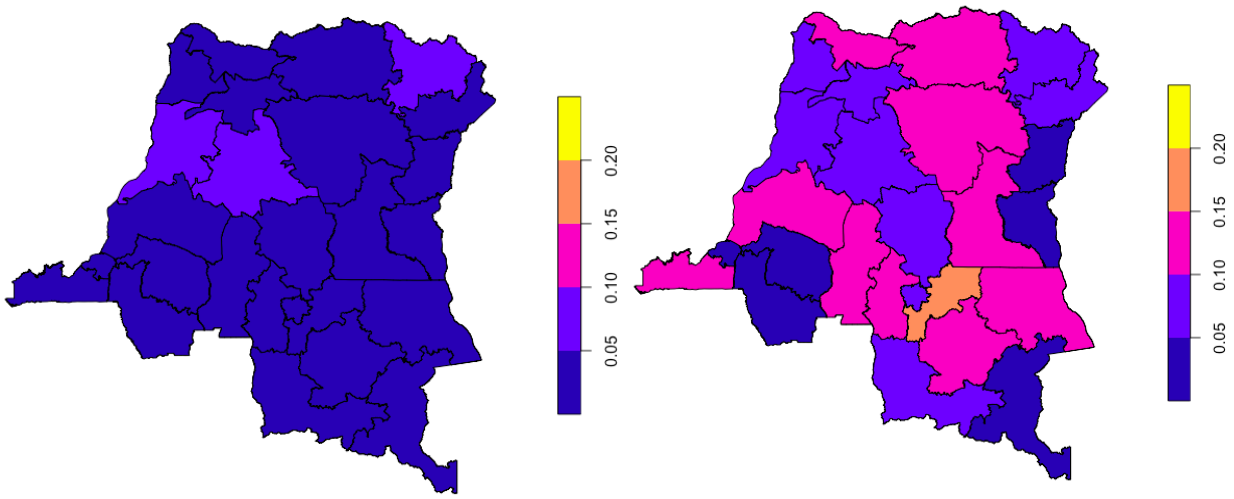


Figure 4.3: Map of patent infection prevalence in 2007 (left) and 2013 (right). Patent infection prevalence changes ranged from a decrease of -0.9% to an increase +12.8%.

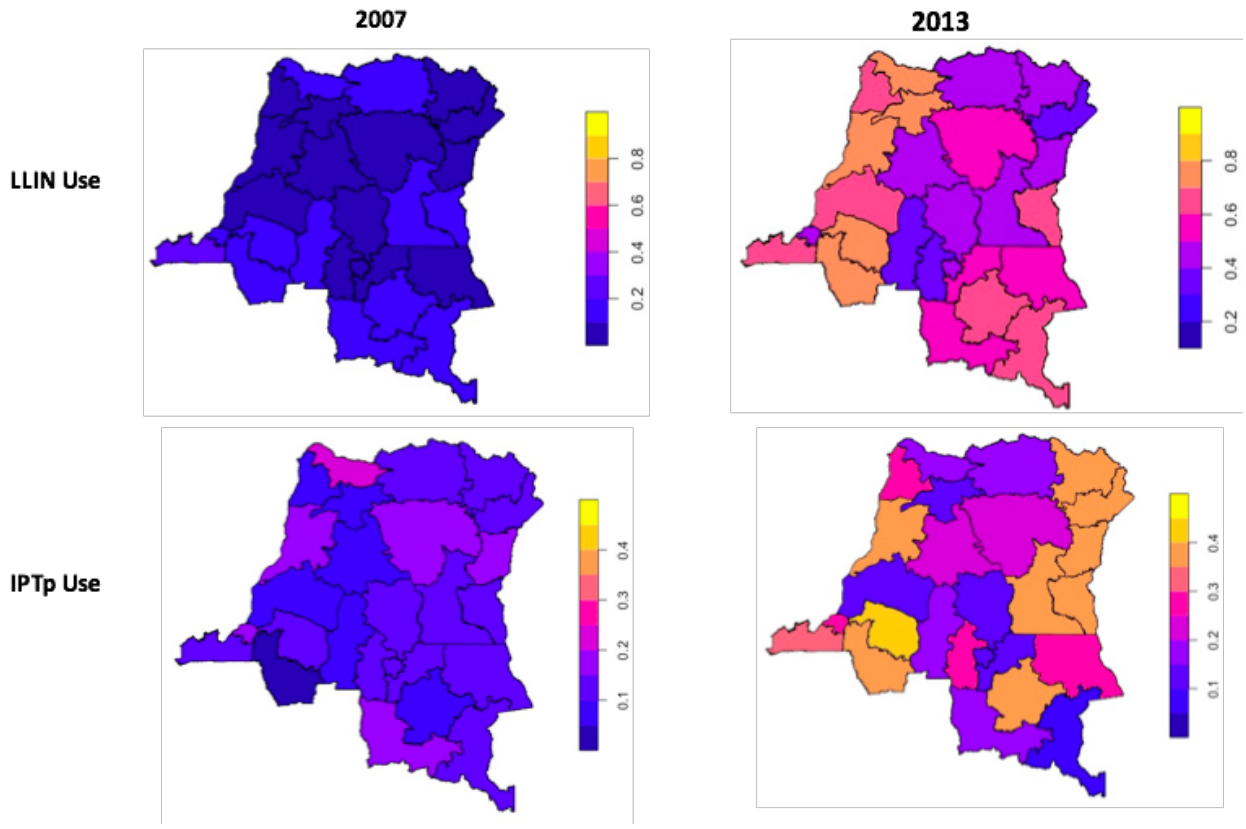


Figure 4.4: The proportions of respondents reporting sleeping under a LLIN and the proportions of women reporting receiving IPTp during most recent pregnancy in 2007 (left) versus (2013). Increases in LLIN and IPTp use were heterogeneous across the DRC.

LLIN modeling results: The best fitting model was a multilevel model with a non-spatial random intercept, adjusted for several confounders (DIC = -364.00) (**Table 4.3**). Thus, the results from this model represent our primary findings; increasing LLIN use was associated with a reduction of only 1.7% (95% CI = -3.2%, -0.3%) in patent infection prevalence between 2007 and 2013 (**Table 4.3**). We did not see a difference in the association when restricting LLIN use to only deltamethrin and alphacypermethrin nets (**Table 4.3**). Results from the spatial model matched those from the non-spatial models (**Supplementary Table B4**).

In the secondary analysis, we found a protective effect of individual LLIN use, though the protection was stronger for those who used deltamethrin and alphacypermethrin nets

(**Supplemental Table B5**). The results of these models indicate that there are differences in the protective effect of individual net use across the country (**Supplementary Figure B2**), though these differences are attenuated when restricting to deltamethrin and alphacypermethrin net use only (**Supplementary Figure B3**). For deltamethrin and alphacypermethrin nets, the effects ranged from approximately OR = 0.50 (SE = 0.20) to OR = 0.83 (SE = 0.16). We found no individual protective effect of permethrin treated nets (**Supplementary Table B6**). Within each province, the proportion of all LLINs used that were treated with permethrin differed from under 5% to approximately 50% (**Supplementary Figure B4**).

Discussion:

In this study, utilizing data from the largest, most recent, and nationally-representative, population-based health surveys conducted in the DRC, we found a three-fold increase in the prevalence of patent *P. falciparum* infections amongst adults between 2007 and 2013. We also found high spatial heterogeneity of the changes in prevalence, with increases as large as 12.8%. Importantly, the map of changes in prevalence does not reflect the regions where health organizations work or where intervention programs were rolled out between 2007 and 2013[131]. These findings indicate that further investment in malaria control is needed in the DRC, particularly in the regions that had the highest increases. These findings support those from other groups that hospitalizations due to malaria increased during this same time period, and that the proportion of individuals testing positive for malaria increased (by RDT and microscopy)[20,130]. While the authors of the latter study suggest that this increase is due to the introduction of RDTs, the findings from the present study support the notion of a true increase in infections.

Though it is possible that the prevalence of patent infections increased between 2007 and 2013 while malaria prevalence overall dropped during this time, the data from this study support

the notion of a true increase in prevalence. As prevalence decreases, we expect a decrease in immunity to infection and thus an increase in the proportion of high-parasitmetic patent infections[69]. Thus, the increase in patent infections that we observed in this study may be a result of an overall decrease in prevalence. However, in this scenario, we would expect to see a shift in the age distribution of those infected, with patent infections occurring amongst older individuals in 2013 compared to 2007[140]. We did not observe any such change in the shape of the age distribution or in the median age of those infected between 2007 and 2013, which again supports the conclusion that prevalence increased during this time-period.

Interestingly, we found that most children with fevers received quinine in both 2007 and 2013, and quinine represented a larger proportion of the of anti-malarial therapeutics distributed between 2007 and 2013. Quinine is not a recommended treatment for malaria in the DRC; Artesunate/Amodiaquine has been the first line recommended treatment since 2005[77]. While these data are from children, it is important to note that nearly two thirds of children with fevers in 2013 received a malaria drug that is not the recommended treatment.

We found that the prevalence of patent infections increased amongst pregnant women. This is particularly concerning, as pregnant women are at risk for developing placental malaria, potentially leading to adverse birth outcomes such as premature delivery or a baby of low birth weight[6,8]. Importantly, we found that amongst women who received any IPTp, the proportion with patent infections was only slightly lower than the proportion amongst women who did not receive IPTp. However, the low number of women who received IPTp limits our ability to make definitive conclusions regarding the efficacy of the IPTp program.

Despite an increase in IPTp use from 2007, overall use remains low, particularly the full course of three SP doses. In 2013, only 13.6% of women reported receiving at least two of the

three recommended doses of SP during their most recent pregnancy. This proportion is far lower than that of other sub-Saharan African countries; 60% of Ghanaian women and 37% of Kenyan women reported receiving all three doses of SP during their previous pregnancy[141]. In the neighboring country of Uganda, the proportion was 25%[141]. Barriers to IPTp uptake from other countries include drug shortages at health facilities, improper training for facility staff, and lack of community knowledge of IPTp programs[142]. The maps of IPTp uptake demonstrate heterogeneous uptake across the DRC, indicating that certain regions likely have better health infrastructure and access to health facilities. This notion is supported by the finding that areas with high LLIN usage also had higher IPTp usage. Additional work is needed in the DRC to understand the factors that are contributing to the low uptake of IPTp and determine strategies for increasing use.

The results of the territory-level LLIN models indicate that increasing LLIN usage was associated with only a small decrease in patent infection prevalence. From these results, we cannot conclude that LLINs did not provide any protection, as it is difficult to determine what the patent infection prevalence in 2013 would have been had LLIN use remained at the 2007 levels. However, it is important to note that despite a large increase in LLIN use between 2007 and 2013, the prevalence of patent infections increased. These findings point to additional factors driving the increase in prevalence, which warrants further study. It is critical to determine whether LLIN use truly provides only a slight protection, or if other factors (such as insecticide resistance) are overwhelming the true protective effect of LLINs. The findings from the present study indicate that additional control measures are necessary to reduce malaria burden amongst adults in DRC.

The finding that increasing LLIN use demonstrates only a slight protective association with patent infections amongst adults is supported by several studies of *Anopheles* biting behavior. A study from Burkina Faso found that *Anopheles* mosquitoes begin biting around sunset, before many adults go to sleep, rendering the bed-net less useful[120]. More concerning is the finding from a study conducted in Papua New Guinea that *Anopheles* biting behavior shifted earlier in the evening after a bed-net distribution campaign[121]. Currently, the DRC does not have sufficient entomological monitoring to determine if this has occurred[20]; however, that adults are exposed to *Anopheles* biting for several hours before they go to sleep may help explain the limited protection from LLINs that we observed in this study. Thus, for adults in particular, who go to sleep later in the evening than children, vector control methods such as IRS may help reduce malaria prevalence.

There are several strengths of this study. We utilized data from the two population-based, national health surveys conducted in the DRC. These surveys include data from over 24,000 individuals. This is the first study, to our knowledge, to evaluate changes in malaria in the DRC amongst adults over time using nationally-representative data. Previous studies, including those from the Malaria Atlas Project, have focused exclusively on children or pregnant women, or were not nationally-representative[16,130]. Thus, this study adds a critical piece to the body of research focused on malaria in the DRC. Additionally, we utilized qPCR data, the most sensitive method for detecting malaria[9].

The findings from this study are subject to limitations. The first is that the intervention data utilized were obtained from self-reports, which is subject to recall bias. To minimize this bias, the DHS asks multiple questions regarding LLIN use. Our analysis of these data indicated no differences amongst the various questions the DHS uses to assess LLIN use. This provides

assurance that the recall bias is low. Additionally, as we only assessed prevalence of patent infections, we were unable to report changes in the overall prevalence of malaria amongst adults. However, as patent infections are those that are detectable by microscopy, this is a clinically meaningful measure for the DRC. Lastly, we utilized ecological models and thus the findings from the territory-level models cannot be interpreted on an individual scale.

Conclusions:

Patent *P. falciparum* infection increased three-fold amongst adults in the DRC between 2007 and 2013-2014. Though use of LLINs and IPTp increased, uptake remains relatively low and further investment is needed to ensure the success of these programs. Additional factors, such as drug and insecticide resistance, could have contributed to the increased prevalence of patent malaria infections. Further scale-up of these programs and new interventions are needed.

Table 4.1: Description of individuals included in the 2007 and 2013-2014 DHS Surveys

	2007 DHS	**2013-2014 DHS**
Number of adults sampled	9,275	18,275
Number of DHS clusters	300	536
Number of individuals with PCR data	9,218	17,934
Number of individuals included in analysis	8,698	16,126
Age range in years	15-59	15-59
Median Age (IQR)	27 (20-35)	28 (20-38)
Percent Female	48	52
% of individuals reporting sleeping under an LLIN	11.9%	54.0%
% of pregnant women reporting receiving any IPTp	15.8%	31.1%
% of pregnant women receiving at least two doses of IPTp	7.3%	13.6%

Table 4.2: Proportion of women pregnant at time of the DHS with patent infections by IPTp status in 2007 and 2013

Survey Year	**2007**			**2013-2014**		
	Received any IPTp	*Did not receive IPTp*	*p-value**	*Received any IPTp*	*Did not receive IPTp*	*p-value**
Total N (%)	49 (9.4)	471 (90.6)		232 (21.8)	830 (78.2)	
N with patent infections (%, SE)	1 (2.1, 2.0)	21 (4.5, 0.1)	0.711	22 (9.5, 1.9)	109 (13.1, 1.2)	0.167
	Received 2 doses of IPTp	*Did not receive 2 doses of IPTp*	*p-value*	*Received 2 doses of IPTp*	*Did not receive 2 doses of IPTp*	*p-value*
Total N (%)	22 (4.4)	498 (95.6)		99 (9.3)	963 (90.7)	
N with patent infections (%, SE)	0 (0, 0)	22 (4.4, 0.1)	0.6411	12 (12.1, 3.3)	199 (12.3, 1.1)	~1.000
	Received 2 doses of IPTp	*Received 1 dose of SP*	*p-value*	*Received 2 doses of IPTp*	*Received 1 dose of SP*	*p-value*
Total N (%)	22 (44.9)	27 (55.1)		99 (42.7)	133 (57.3)	
N with patent infections (%, SE)	0 (0, 0)	1 (3.7, 3.6)	~1.000	12 (12.1, 3.3)	10 (7.5, 2.3)	0.339

*p-value for the chi-squared and Fisher's exact tests of the null hypotheses that there is no difference in proportions

Table 4.3: Results of territory-level multilevel models of LLIN use on change in patent prevalence. The exposure for each model is proportion of adults reporting using an LLIN, the outcome is change in territory-level prevalence between 2007 and 2013. The beta coefficients represent the change in patent prevalence associated with an increase in territory level net use

Exposure	**Beta**	**95% CI**	**DIC**	**Beta**	**95% CI**	**DIC**
	Crude			*Adjusted**		
Proportion of LLIN use (all nets)**	-0.017	-0.031, -0.003	-321.84	-0.017	-0.031, -0.003	-364.00
Proportion of Deltamethrin and Alphacypermethrin bed-net use**	-0.013	-0.021, -0.005	-327.11	-0.011	-0.020, -0.003	-366.53

*models are adjusted for territory-level patent prevalence in 2007, net use in 2007, average education level, and urbanicity score
**logit transformed

CHAPTER FIVE: CONCLUSION

Summary of Aims:

In this dissertation, I sought to assess the current state of malaria amongst adults in the DRC. Despite being home to the second highest burden of disease in the world, there are few nationally-representative studies of malaria in the DRC. The only nationally-representative surveys of adults in the Congo were conducted in 2007 and 2013-14. Several studies have been conducted amongst children under the age of five in the DRC, describing the high national prevalence of infections (38.6%), areas of highest prevalence, and risk factors for infection[2,9,116]. However, no such studies have been conducted amongst adults in the DRC; understanding malaria epidemiology amongst adults is critical, as discussed in the Chapter Two. Thus, the overarching goals of this dissertation were to understand who is infected, where are people infected, and how effective are the interventions that the DRC has in place to reduce prevalence.

The Aims of this dissertation were formulated to address these questions. In Aim 1, I evaluated the prevalence of spatial distribution of infections, as well as risk factors for infection. I also sought to understand whether these risk factors differed between urban areas versus rural areas. We found a high national prevalence, ~30%, and a high amount of spatial heterogeneity amongst those infected. Additionally, we identified several individual, household, and community-level risk factors, which will help the DRC detect people and groups more likely to be infected. Several risk factors demonstrated modification by urban status, indicating the need to tailor intervention programs to urban versus rural areas. In Aim 2, I investigated changes in malaria prevalence between 2007 and 2013 and assessed whether malaria control programs have

helped reduce malaria prevalence. Over the past two decades, the DRC scaled up investment in two main intervention program, LLINs and IPTp. However, no studies have specifically evaluated the effects of these programs amongst adults. We found that the prevalence of patent infections increased from 2.4% to 7.5%, though within each province, prevalence increased as much as 12.8%. Importantly, increasing LLIN use was not associated with lower patent prevalence. While LLIN use increased, a result of the many LLIN distribution campaigns over the past decade, only about half of adults reported sleeping under a net in 2013. Similarly, IPTp use was low; the MoH must invest in scale-up of IPTp to prevent infections amongst pregnant women. The findings from Aim 2 indicate that the state of malaria amongst adults in the DRC has gotten worse over the past decade, despite increased investment in control strategies.

Conclusions:

The findings from this dissertation highlight the dire state of malaria in the DRC and the need to re-evaluate current control strategies. This is highly concerning considering the many years of work and millions of dollars that the DRC has invested in malaria control programs[20]. Further work, discussed below, is needed to understand barriers to intervention uptake and ensure that all adults have access to LLINs and pregnant women to IPTp. The DRC may benefit from investing in additional intervention programs, many of which have been successful in other countries. These programs include seasonal malaria chemoprevention, intermittent preventative therapy for infants (IPTi), and IRS[143]. Introducing these programs will require an additional financial investment and increased infrastructure. However, the evidence from this dissertation indicates that such programs may be necessary.

Interestingly, the findings from our studies do not confirm those presented by the Malaria Atlas Project (MAP), which indicated a steep drop in prevalence of malaria amongst children

between 2000 and 2017[144]. These differences are not surprising given the high uncertainty of the MAP estimates[144]. Though MAP utilizes sophisticated modeling techniques, the lack of nationally-representative studies of children in the DRC limits the ability to make inferences regarding national prevalence estimates over time. Between 2000 and 2017 there has only been one such study of children in the DRC, the 2013-2014 DHS, which included approximately 8,000 children. In the present studies, we analyzed data from over 20,000 adults, at two time-points. Thus, this dissertation fills a critical gap in the literature regarding malaria prevalence in DRC.

Large-scale systemic factors, though not directly related to malaria control, likely contribute to the high burden of disease and limited progress over the past decade. The DRC suffers from political instability, regional violence, and widespread poverty, all of which undoubtedly impact the current state of malaria control[145]. These factors lead to low economic growth and inadequate health infrastructure, which limits the utility of malaria intervention programs[66]. This makes planning malaria control efforts more challenging as they all must be considered within the current political and economic context. Question that must be considered include whether it is possible for the DRC to implement a national IPTi program given the current state of health infrastructure and how to ensure that malaria control programs reach people living in conflict zones. It is not surprising that systemic factors influence malaria transmission. Historically, economic development and modernization both helped eliminate malaria in the United States[146]. Similarly, as discussed in this dissertation, urbanization contributes to large reductions in malaria prevalence worldwide[57,58]. The importance of such broader factors indicates that though malaria intervention programs are useful, large-scale

structural changes such as economic growth and infrastructure development are necessary for ensuring sustained malaria control in the DRC.

This dissertation fills a critical gap in the literature by adding insight into the epidemiology of malaria amongst adults and adds to the body of work regarding malaria epidemiology in the DRC. The findings will hopefully aid malaria control programs, both in the DRC and other sub-Saharan countries. The findings and conclusions should also serve as a foundation for additional studies in the DRC.

Future Directions*:*

There is certainly more work that must be done to combat malaria in the DRC. Evaluating programmatic data from groups such as PMI and SANRU will help further our understanding of the effects of the intervention programs. These data were not available for these studies and thus I relied on self-reported data. However, future studies should aim to determine the impact and effectiveness of the LLIN and IPTp distribution programs, in addition to the effect of LLIN and IPTp use itself. While national surveys such as the DHS are critical tools for assessing the effects of intervention use, programmatic data will provide an additional perspective to help improve malaria control policies[147]. Quantifying the number of LLINs distributed within each territory and the number of doses of IPTp delivered to each health zone will allow the MoH to identify areas in greatest need of help.

Further work utilizing qualitative methods will help shed light on LLIN and IPTp use patterns. Understanding behaviors regarding intervention use or barriers to use is necessary to develop strategies for increasing use. As mentioned in Chapter Four, various barriers, such as lack of access to health facilities and insufficient training for healthcare providers, have been reported in other sub-Saharan African countries. Interviews and focus groups with community

members, health workers, and members from PMI and SANRU will provide necessary insight and points of view to help elucidate what factors are hindering intervention uptake in the DRC.

Regarding Aim 1, determining the causal effects of the identified risk factors will further our understanding of the mechanisms driving malaria risk. The purpose of this study was to identify marginal associations, not causal effects. Though marginal associations are useful for identifying individuals or groups at higher risk for infection, they are not necessarily useful for informing intervention programs or policy. However, the associations identified in this study present avenues for future work. Specifically, the protective association of modern housing and metal roofing should be further explored using causal inference methods. A protective causal effect would indicate that housing improvements can be used as a method for reducing malaria risk. Thus, the findings from Aim 1 should serve to generate questions and hypotheses for future studies.

Regarding Aim 2, future studies should aim to further assess factor contributing to changes in malaria prevalence such as environmental changes. In Aim 2, I assessed LLIN and IPTp use only; however, changes in temperature or rainfall between 2007 and 2013 have also likely played a role in the differences in prevalence that we observed. These factors are known to be associated with malaria risk and should be explored in future studies[3]. As the effects of climate change become more and more evident, it is critical to understand the role of these changes on malaria risk in the DRC. Mosquito breeding seasons may shift or last longer, affecting the seasonality of malaria risk, or increasing temperatures may cause reduced breeding and thus reduce risk in certain areas[148,149]. Understanding the role of these changes in the DRC may shed light on which intervention programs are likely to be more effective (i.e.: implementing IRS or introducing seasonal chemoprevention programs). The findings from the

Aim 2 indicate that there remain unstudied factors that lead to an increase in the prevalence of patent infections amongst adults, despite increased LLIN use. Changing climate may be one of these factors and should be further explored.

Overall, this dissertation serves to underscore the importance of studying malaria amongst individuals of all ages and not only focusing on children under the age of five. Including all age groups in future DHS and Malaria Indicator Cluster Surveys (MICS) in the DRC, and specifically including malaria testing, will provide critical data to improve control efforts. Consistent, comparable data are necessary for monitoring the progress of malaria control programs in the DRC. While most deaths due to malaria occur amongst children, the findings from this dissertation highlight the high burden amongst adults. A currently on-going MICS in the DRC again only includes children and thus we will not have updated data for adults. While including all age groups is more operationally challenging than focusing only on children, it will provide necessary information that will help the MoH combat malaria.

APPENDIX A: CHAPTER THREE SUPPLEMENTARY MATERIALS

Modern Housing variable construction: We constructed a composite "modern housing" variable using data from the roof, wall, and floor material variables reported in the DHS. The definition of modern housing was based off those used by Tusting et al[96]. Modern roofing materials included: metal, zinc/cement, tiles/slate, or cement. Modern wall material included: cement, stone, bricks, or covered adobe. Modern floor materials included: vinyl, asphalt, ceramic tiles, cement, or carpet. Only houses with a modern roof, walls, and floor materials were considered "modern housing".

PrevMap analysis: We estimated cluster-level drug resistance allele frequencies using the *PrevMap* package in R[105]. We fit a model in order to generate cluster-level SNP prevalence estimates at all sampled DHS clusters from the 1,065 children with available data. Each resistance mutation was analyzed individually. We first determined raw cluster-level SNP frequencies and then transformed the proportions using a logit transformation. We fit linear a geospatial model of the following form:

$$yi = S(xi) + Zi$$

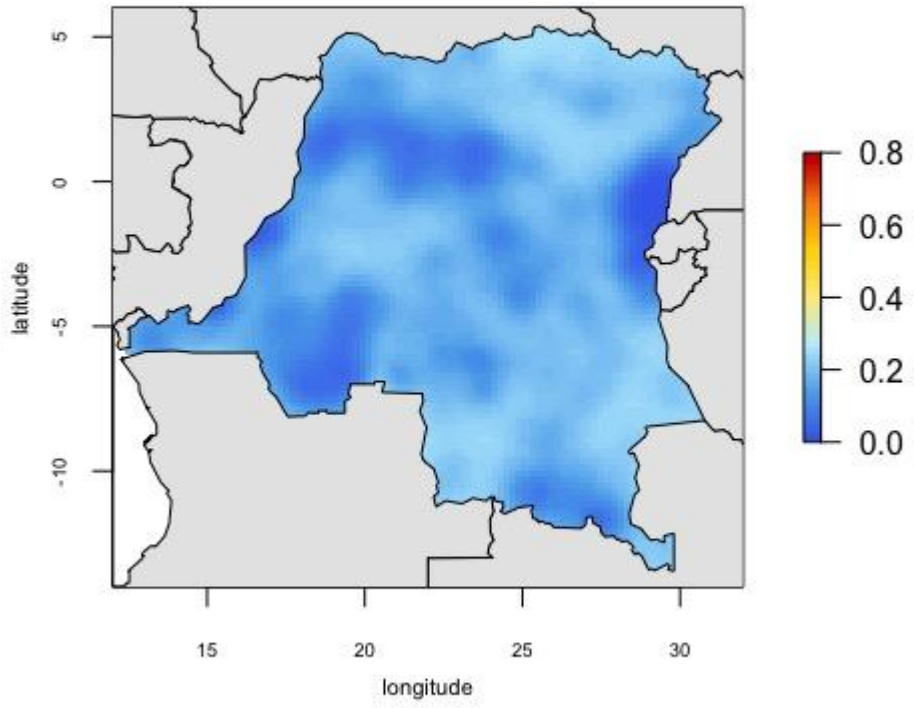
In this model, yi is equal to the transformed allele frequency for each cluster and $S(xi)$ represents an isotropic Gaussian Process with a variance of $\boldsymbol{\sigma}^2$ and a Matern correlation function[105]. Zi represents a Gaussian error term[105]. Model parameters were estimated using maximum likelihood and models were run using 10,000 simulations to generate spatially smooth frequency estimates. After fitting the model, we extracted the frequency estimate for each DHS

cluster included in the analysis. To minimize bias, we averaged the estimated values for all geopoints within 15km square of the DHS cluster geopoint.

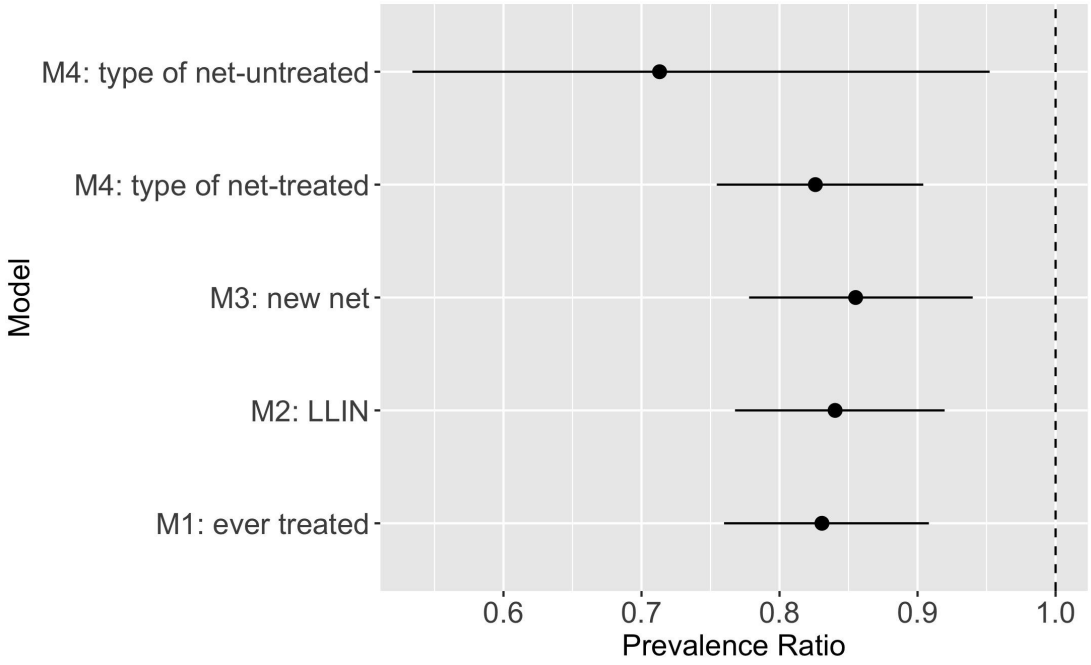
We used the same model framework to generate spatially smoothed *P. falciparum* prevalence estimates (**Figure 3**). Using this model, we also determined the estimate error for all points (**Supplementary Figure A1**).

Bed-net use sensitivity analysis: We evaluated four different methods for determining bed-net usage based on the questions asked in the DHS questionnaire. The first (M1) asked if the individual slept under an "ever treated" net the previous night. The second (M2) asked if the individual slept under a "long lasting insecticide treated net". For the third method (M3), we constructed a "new net" variable based on whether the individual had obtained the net, or re-treated it with insecticide, within the previous 3 years[96]. Lastly (M4), the DHS asks, "the type of mosquito bed-net person slept under", with options of no net, an untreated net, or a treated net. We compared the estimated prevalence ratio of those who used nets versus those who did not based on each method's definition of net use. The results (**Figure A2**) demonstrate no substantial differences in the effect of net use between the four methods. The variable used in M1 was used for the primary analysis.

Appendix A Figures and Tables:



Supplementary Figure A1: Standard error map for smoothed *P. falciparum* PCR prevalence estimates generated using *PrevMap*[105]



Supplementary Figure A2: Results of sensitivity analysis evaluating different methods for measuring and coding bed-net usage. Each model compared the prevalence of PCR detectable *P. falciparum* amongst individuals who reported using a net versus those who did not.

Supplementary Table A1: Province level *P. falciparum* prevalence estimates measured by PCR.

Province name	Prevalence % (SE)
Kinshasa	18.6 (2.3)
Kwango	21.4 (3.7)
Kwilu	25.8 (5.0)
Mai-Ndombe	43.9 (4.7)
Kongo Central	40.5 (3.8)
Equateur	22.4 (2.7)
Mongala	26.3 (2.9)
Nord-Ubangi	48.9 (5.0)
Sud-Ubangi	31.3 (5.3)
Tshuapa	33.7 (4.4)
Kasai	44.5 (7.3)
Kasai-Central	43.5 (4.0)
Kasai-Oriental	35.9 (4.3)
Lomami	52.5 (3.1)
Sankuru	27.2 (3.3)
Haut-Katanga	22.5 (3.4)
Haut-Lomami	41.0 (5.2)
Lualaba	38.6 (9.6)
Tanganyka	54.3 (5.1)
Maniema	45.5 (5.7)
Nord-Kivu	6.7 (1.6)
Bas-Uele	58.3 (6.4)
Haut-Uele	42.7 (3.6)
Ituri	33.9 (4.1)
Tshopo	29.6 (4.9)
Sud-Kivu	16.4 (3.2)

Supplementary Table A2: Descriptive statistics of individuals with missing GPS data. Values are unweighted as sampling weights are not assigned to individuals missing location data.

	PCR positive	**PCR negative**
Total (unweighted)	607	795
Individual-level		
Age	27 (20 - 36)	30 (22 - 39)
Female (%)	313 (51.6%)	412 (51.8)
HIV positive (%)	8 (1.3)	9 (1.1)
Education		
No School	78 (12.9)	131 (16.5)
Primary	262 (43.2)	319 (40.1)
Secondary	259 (42.7)	337 (42.4)
Higher than secondary	8 (1.3)	8 (1.0)
Owns a bed-net	443 (73.0)	603 (75.6)
Slept under a bed-net	325 (53.4)	459 (57.7)
Wealth (%)		
Poorest	197 (32.5)	270 (34.0)
Poor	164 (27.0)	200 (25.2)
Middle	159 (26.2)	199 (25.0)
Rich	79 (13.0)	112 (14.1)
Richest	8 (1.3)	14 (1.8)
Household-level		
Average number of bed-nets per person (SE)	0.27 (0.01)	0.27 (0.01)
Modern Housing (%)	8 (1.3)	11 (1.4)
Metal Roofing (%)	41 (6.8)	82 (10.3)
Cluster-level		
Median Age (IQR)	29 (27- 30.5)	28 (26 – 30.5)
Median Wealth (IQR)	2 (2-3)	2 (2-3)
Median Education (IQR)	2 (2-3)	2 (2-3)

Supplementary Table A3: Associations between identified risk factors and *P. falciparum* prevalence, stratified by urbanicity

Variable	Urban Category	Prevalence Ratio	95% Confidence Interval	P-value	F-test
Individual-level:					
Bed-net use (all brands)	Rural	0.79	0.71 – 0.89	<0.001	0.442
	Urban	0.86	0.73– 1.01	0.066	
Deltamethrin or alphacypermethrin bed-net use	Rural	0.75	0.66- 0.85	<0.001	0.077
	Urban	0.94	0.77- 1.14	0.523	
Female Sex	Rural	0.83	0.77 – 0.90	<0.001	0.909
	Urban	0.84	0.75– 0.94	0.002	
Age (scaled)	Rural	0.86	0.83 – 0.90	<0.001	0.537
	Urban	0.84	0.78– 0.90	<0.001	
Modern Housing	Rural	0.98	0.71 – 1.34	0.876	0.035
	Urban	0.60	0.46 – 0.79	<0.001	
Metal Roofing	Rural	0.85	0.69– 1.03	0.099	0.002
	Urban	0.53	0.43- 0.66	<0.001	
Wealth	Rural	0.95	0.90– 1.01	0.106	<0.001
	Urban	0.72	0.65– 0.80	<0.001	
Education	Rural	1.03	0.96 – 1.11	0.435	<0.001
	Urban	0.80	0.71– 0.89	<0.001	
Net Ratio >0.5	Rural	0.82	0.72 - 0.93	0.002	0.521
	Urban	0.90	0.72- 1.12	0.351	

Cluster-level:					
Deltamethrin or alphacypermethrin net use*	Rural	0.93	0.89 – 0.97	0.002	0.040
	Urban	1.07	0.94– 1.21	0.299	
SP† use	Rural	0.96	0.94- 0.97	<0.001	0.458
	Urban	1.00	0.87 - 1.15	0.953	
A437G*	Rural	0.90	0.85 – 0.95	<0.001	0.599
	Urban	0.92	0.85 – 0.99	0.032	
K540E*	Rural	0.96	0.92 – 0.99	0.009	0.180
	Urban	0.92	0.87 – 0.97	0.002	
A581G*	Rural	0.85	0.78 – 0.92	0.001	0.507
	Urban	0.80	0.67– 0.94	0.008	
CRT K76T*	Rural	0.95	0.92 - 0.98	<0.001	0.649
	Urban	0.93	0.87 - 0.99	0.029	

† Sulfadoxine/pyrimethamine
*logit transformed

Supplementary Table A4: Comparison of the association between individual LLIN use vs no LLIN[†] use and malaria prevalence between adults and children in the 2013-2014 Demographic and Health Survey. Data from children has been previously published[2,9].

Population	Prevalence Ratio	95% Confidence Interval
Children	0.82	0.72 – 0.91
Adults	0.83	0.76 – 0.91

[†]Long-lasting insecticide treated net

APPENDIX B: CHAPTER FOUR SUPPLEMENTARY MATERIALS

*Prior distributions for spatial models:*We adopted the following priors for the spatial models. For beta coefficients, we adopted standard normal priors. For the phi spatial parameter we used a global conditional autoregressive prior, as proposed by Leroux et al[83]. This was specified as:

$$\phi_k | \phi_{-k} \sim N\left(\frac{\sum_{i=1}^{n} \omega_{ki}\,\phi_i}{\sum_{i=1}^{n} \omega_{ki} + 1 - \rho}, \frac{\tau^2}{\sum_{i=1}^{n} \omega_{ki} + 1 - \rho}\right)$$

Here, wki represents an indicator for whether the area is a neighbor or area k. Thus, the spatial parameter is weighted according to the area-level neighbor matrix. Rho is the spatial autocorrelation parameter and can vary from 0, indicating no spatial autocorrelation, to 1, indicating strong spatial autocorrelation. We chose to not specify rho but rather model the parameter itself and provide a uniform prior(0,1). For the tau variance parameter we assigned an inverse-gamma prior IG(2,1), as has been adopted previously for spatial models of malaria[3].

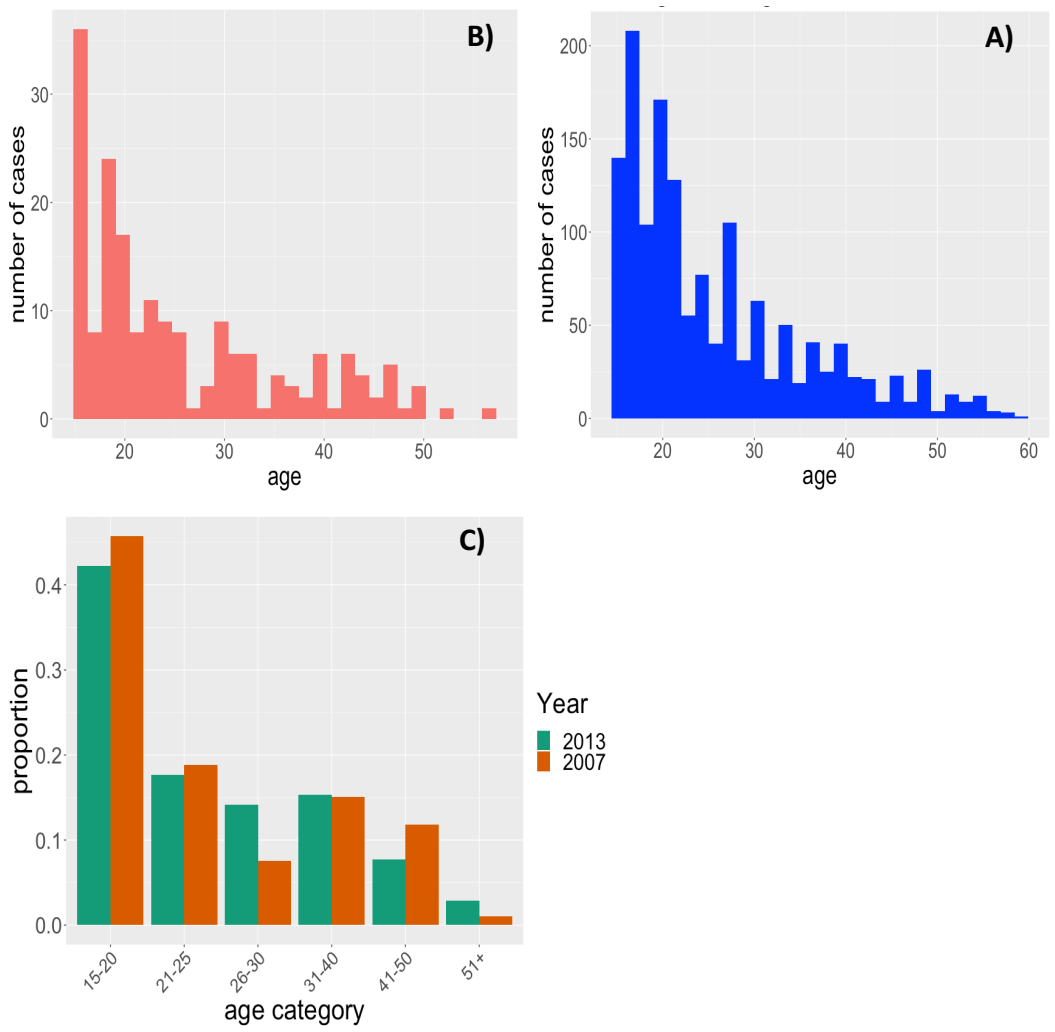
Multilevel model specification: We used multi-level modeling to estimate the effect of individual LLIN use within each province. The model was of the following form:

$$Pr(Yhi) = \boldsymbol{\beta}0 + \boldsymbol{\beta}(LLIN\ use) + \boldsymbol{\beta}Xhi + u0h + u1(LLIN\ use) + ehi$$

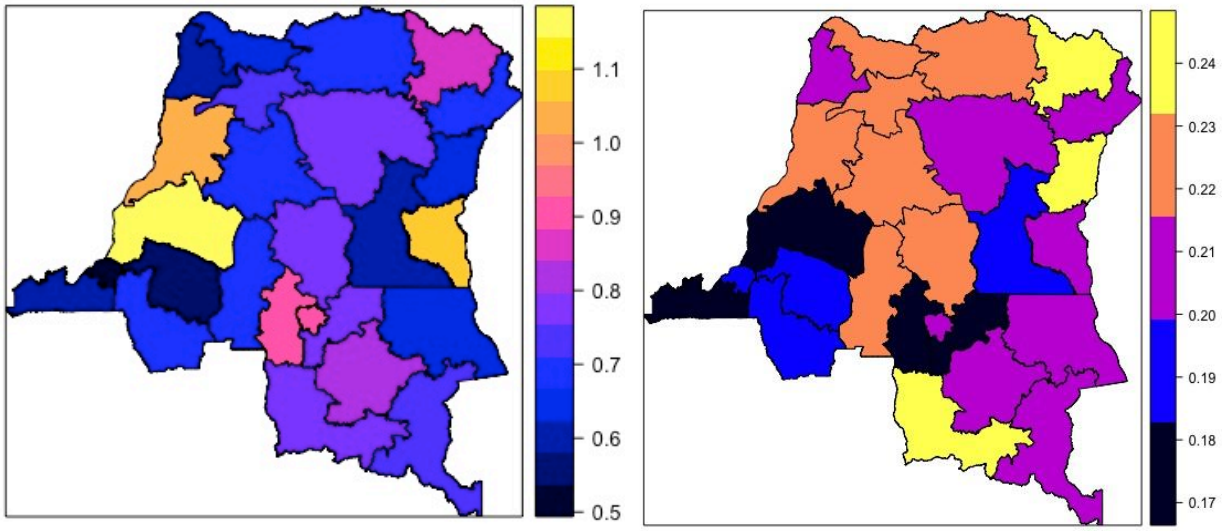
$$\mu_{0h} \sim N(0, \sigma^2_{\mu 0})$$
$$\mu_{1h} \sim N(0, \sigma^2_{\mu 1})$$
$$e_{hi} \sim N(0, e_0^2)$$

In this model, $Y$is a binomial indicator for whether the individual had a patent infection. $B(LLIN\ use)$ is a fixed effect for LLIN use and $BXhi$ is a vector of covariates. $u0h$ represents a random intercept for each province, and $u1(LLIN\ use)$ represents a random slope for LLIN use. ehi represents random error for each individual.

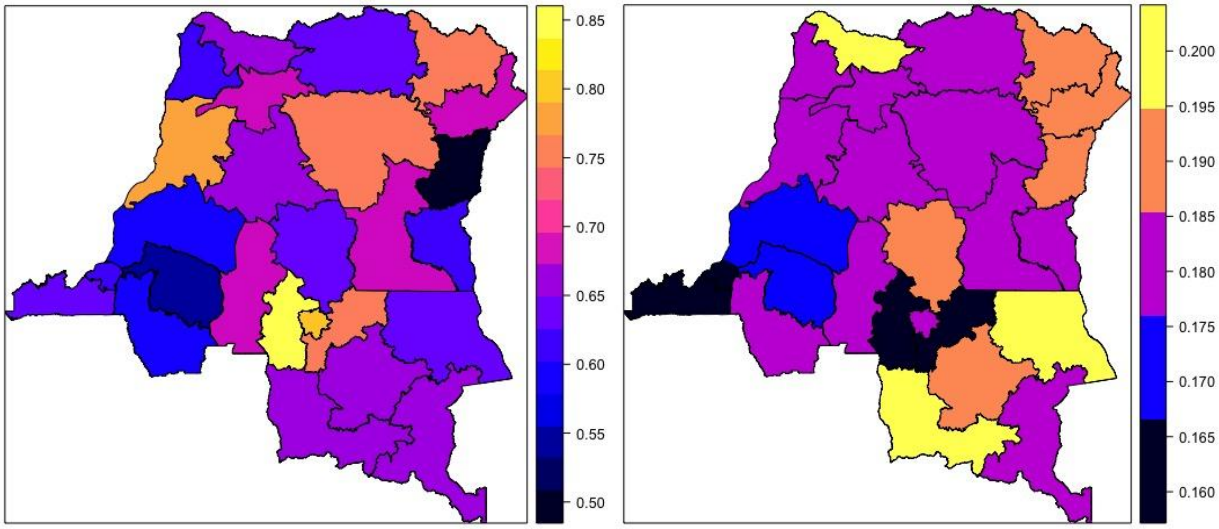
Appendix B Figures and Tables:



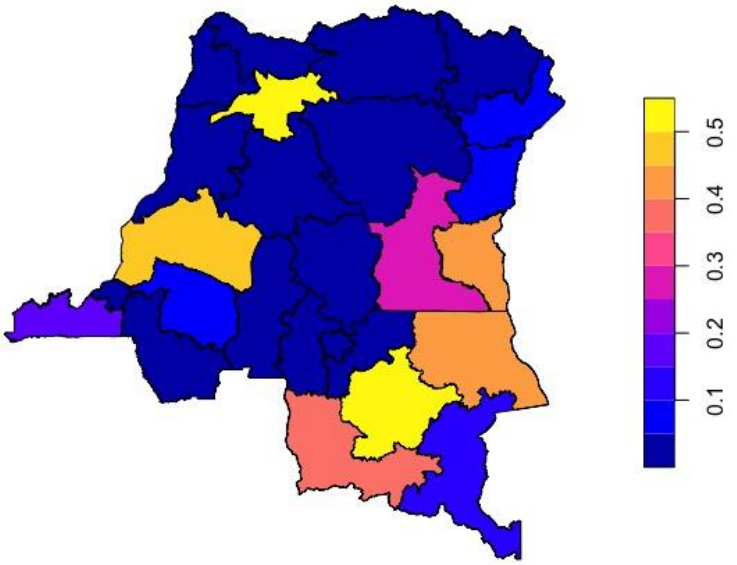
Supplementary Figure B1. Age distributions of patent infections in 2007 (A), 2013 (B) and the prorportion of total infections in each age group between 2007 and 2013 (C). We do not see a large shift in the age distribution over time. The median age of infection in was 21.5 (IQR = 18 - 31) in 2007 and 22 (IQR = 18-31) in 2013.



Supplementary Figure B2: Odds ratios (left) comparing effect individual-level net use versus no LLIN use on odds of malaria infection. The model is adjusted for province-level net use in 2007, province-level patent *P.f.* prevalence in 2007, and cluster-level urban score. Effect standard errors are presented on the right.



Supplementary Figure B3: Odds ratios (left) comparing the effect of individual-level deltamethrin and alphacypermethrin net use versus no LLIN use on odds malaria infection. The model is adjusted for province-level net use in 2007, province-level patent *P.f.* prevalence in 2007, and cluster-level urban score. Effect standard errors are presented on the right.



Supplementary Figure B4: Proportion of all LLINs used that were treated with permethrin.

Supplementary Table B1: Comparison of 161 samples included in the comparison of the 18S and *pfldh* PCR assays. The initial PCR assays had 81% agreement between the assays (i.e.: 81% of PCR calls were the same between the two assays).

	PCR positive by 18S	**Negative by 18S**
PCR positive by *pfldh*	40	0
Negative by *pfldh*	30	91

Supplementary Table B2. Comparison of 161 samples included in the comparison of the 18S and *pfldh* PCR assays using the 100 parasites/uL of blood cutoff to define patent infections. Using this cutoff, there was 97% agreement between the assays in determining patent infections.

	Patent cases by 18S	**Negative by 18S**
Patent cases by *pfldh*	8	3
Negative by *pfldh*	1	149

Supplementary Table B3: Prevalence of patent infections for each province in 2007 and 2013

Province	2007 Prevalence (SE)	2013 Prevalence (SE)
Kinshasa	1.8 (0.5)	4.7 (0.8)
Kwango	3.5 (1.2)	4.3 (1.5)
Kwilu	1.6 (0.6)	5.2 (1.9)
Mai-Ndombe	2.0 (0.9)	10.8 (1.8)
Kongo Central	1.2 (0.5)	11.2 (2.0)
Equateur	6.6 (1.8)	5.7 (1.0)
Mongala	0.9 (1.0)	6.5 (1.6)
Nord-Ubangi	0.0 (0.0)	10.3 (1.9)
Sud-Ubangi	1.9 (0.7)	7.5 (2.7)
Tshuapa	5.6 (0.3)	9.6 (1.8)
Kasai	1.6 (0.6)	14.4 (3.5)
Kasai-Central	2.6 (0.8)	12.2 (1.5)
Kasai-Oriental	1.5 (0.6)	7.3 (1.5)
Lomami	3.3 (1.3)	15.1 (0.9)
Sankuru	4.1 (2.3)	5.8 (1.3)
Haut-Kantanga	1.1 (0.6)	4.6 (1.4)
Haut-Lomami	1.5 (0.9)	10.6 (2.2)
Lualaba	2.1 (1.8)	9.5 (3.9)
Tanganyka	2.8 (1.2)	11.8 (2.4)
Maniema	2.0 (1.2)	11.5 (3.2)
Nord-Kivu	1.1 (0.5)	1.2 (0.6)
Bas-Uele	2.7 (0.5)	11.4 (4.0)
Haut-Uele	5.7 (2.0)	11.0 (2.0)
Ituri	3.1 (1.8)	9.5 (2.9)
Tshopo	4.0 (2.3)	11.2 (4.2)
Sud-Kivu	0.8 (0.4)	3.3 (0.8)

Supplemental Table B4: Description of anti-malarial drug use amongst children under the age of 5 that reported having a fever in the two weeks prior to the survey.

	2007	***2013***
Total Febrile	1,856	3,544
Received any anti-malarial (%):	552 (29.7)	998 (28.2)
Anti-malarial received (%):		
Fansidar	46 (8.3)	63 (6.3)
Chloroquine	119 (21.6)	22 (2.2)
Amodiaquine	86 (15.6)	79 (7.9)
ACTs*	20 (3.6)	158 (15.8)
Quinine	281 (51.1)	676 (67.7)

*Artemisinin combination therapies

Supplementary Table B5: Results of conditional autoregressive spatial territory-level models assessing effect LLIN use on change in patent prevalence.

Exposure	Beta	95% CI	DIC	Beta	95% CI	DIC
	Crude			*Adjusted**		
Proportion of LLIN use (all nets)**	-0.020	-0.034, -0.005	-316.15	-0.021	-0.036, -0.005	-325.99
Proportion of Deltamethrin and Alphacypermethrin bed-net use**	-0.013	-0.022, -0.004	-319.55	-0.012	-0.020, -0.002	-322.36

*models are adjusted for territory-level patent prevalence in 2007, net use in 2007, average education level, and urbanicity score
**logit transformed

Supplementary Table B6: Effect of LLIN use (individual and cluster-level) on individual odds of patent infection. The outcome for these models is individual odds of patent infection amongst individuals in 2013.

Exposure	Beta	OR	95% CI*	Beta	OR	95% CI*
	Crude			*Adjusted**		
All LLIN insecticides:						
Individual LLIN use	-0.292	0.751	0.656 – 0.851	-0.290	0.748	0.656 - 0.853
Cluster-level LLIN use	-0.065	0.937	0.879 – 0.989	-0.068	0.934	0.882 – 0.990
Deltamethrin and Alphacypermethrin nets only:						
Individual LLIN use	-0.385	0.681	0.593 - 0.780	-0.391	0.677	0.590 - 0.776
Cluster-level LLIN use	-0.117	0.890	0.848 - 0.934	-0.116	0.891	0.849 - 0.934
Permethrin nets only:						
Individual LLIN use	0.052	1.053	0.821 - 1.351	0.049	1.050	0.819 - 1.347
Cluster-level LLIN use	0.107	1.113	0.757 - 1.639	0.124	1.136	0.769 - 1.665

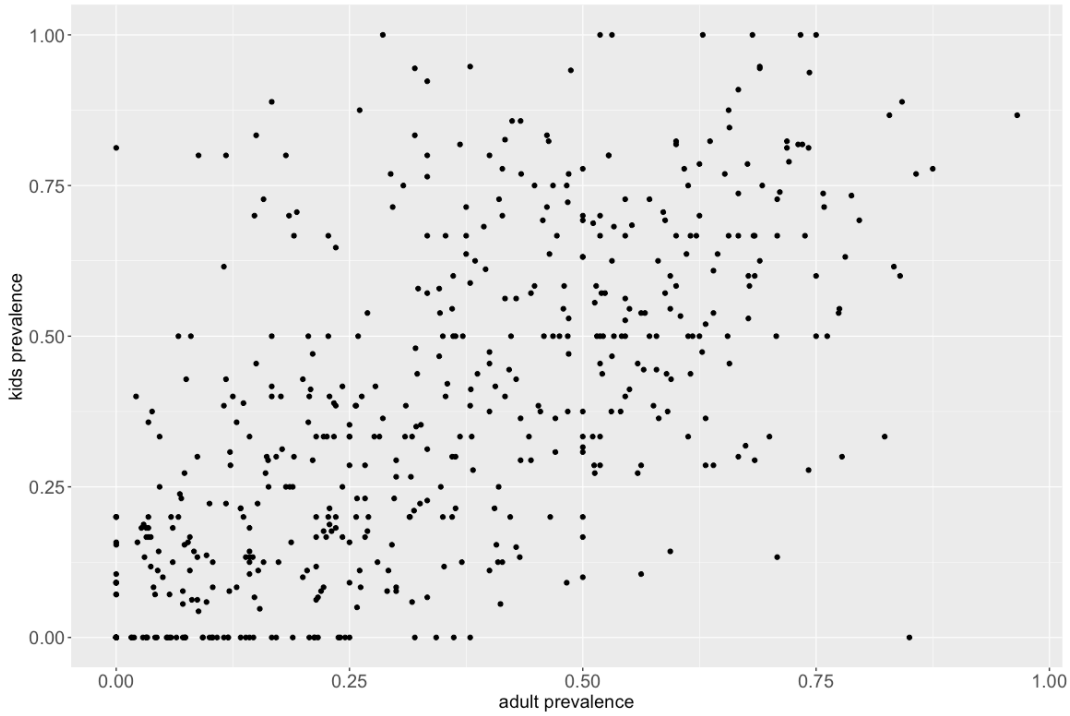
*adjusted for province-level 2007 patent infection prevalence, 2007 province-level of net use, average urbanicity score, and individual wealth category

APPENDIX C: COMPARISON OF PREVALENCE ESTIMATES AMONGST ADULTS VERSUS CHILDREN IN THE 2013-2014 DHS.

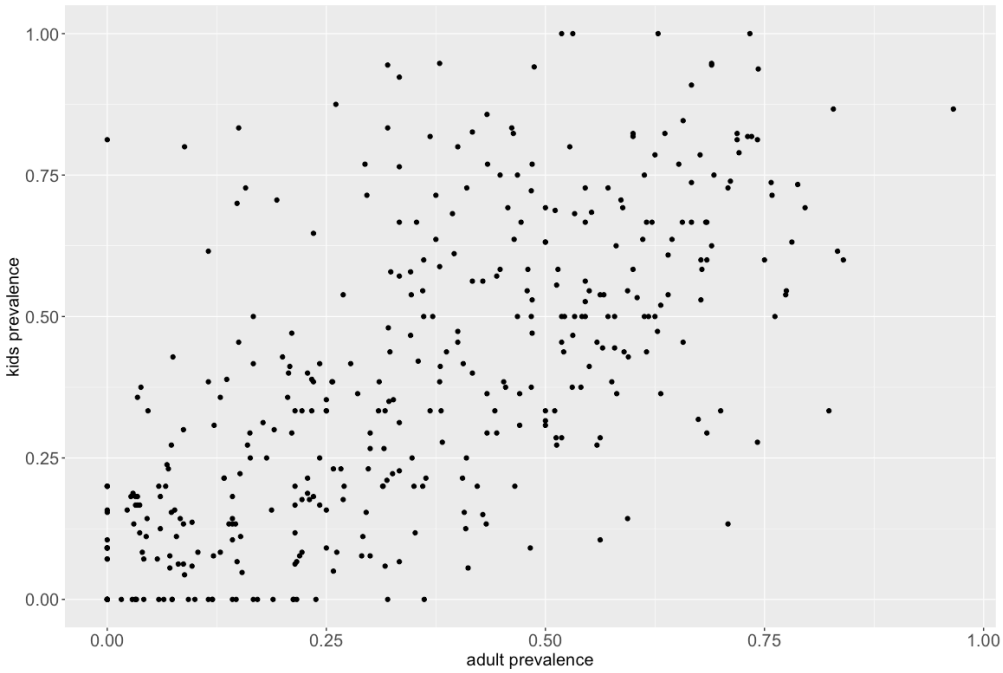
We examined the relationship between the cluster-level prevalence amongst adults versus the prevalence amongst children in the 2013-2014 DHS. The correlation is 0.63 (**Supplementary Figure C1**). In most clusters (202/489) clusters, prevalence was approximately equal between groups (i.e.: within prevalence estimates amongst adults and children were within 10% of each other). In 169 clusters, prevalence was more than 10% higher amongst children than amongst adults. One-hundred and seventeen clusters had a prevalence amongst adults that was more than 10% higher than amongst children. The largest outliers may be due to low sample sizes amongst children in some clusters. When restricting to only clusters with at least 10 adults and 10 children (394 clusters), the correlation is 0.66 (**Supplementary Figure C2**). There does not appear to be any spatial pattern to the clusters with high prevalence amongst adults, defined as prevalence amongst adults 25% or higher than prevalence amongst children (**Supplementary Figure C3**). Clusters with higher prevalence amongst adults should be explored further.

At the province level, the correlation between adult's and kid's prevalence is 0.91. In one province, Kasai, the prevalence amongst adults is 10% higher than amongst kids (**Supplementary Figure C4**). In six provinces, the prevalence amongst children was more than 10% higher than that amongst adults.

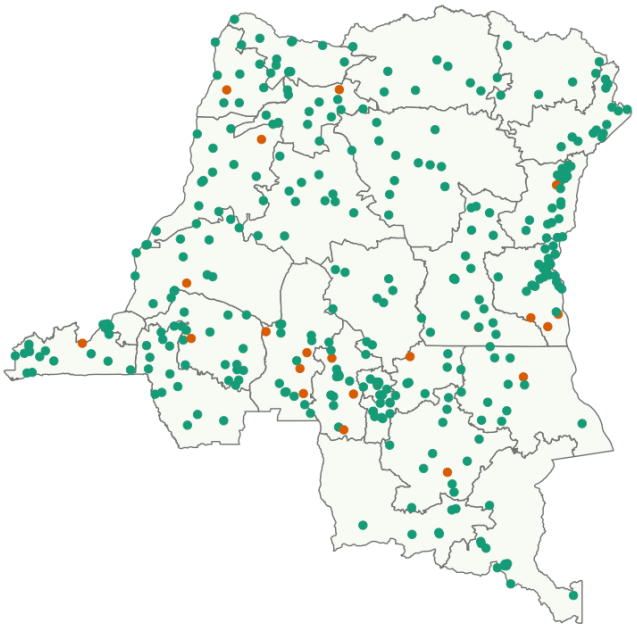
It must be noted that fundamental differences in assay performance between kids and adults limit our ability to make these comparisons.



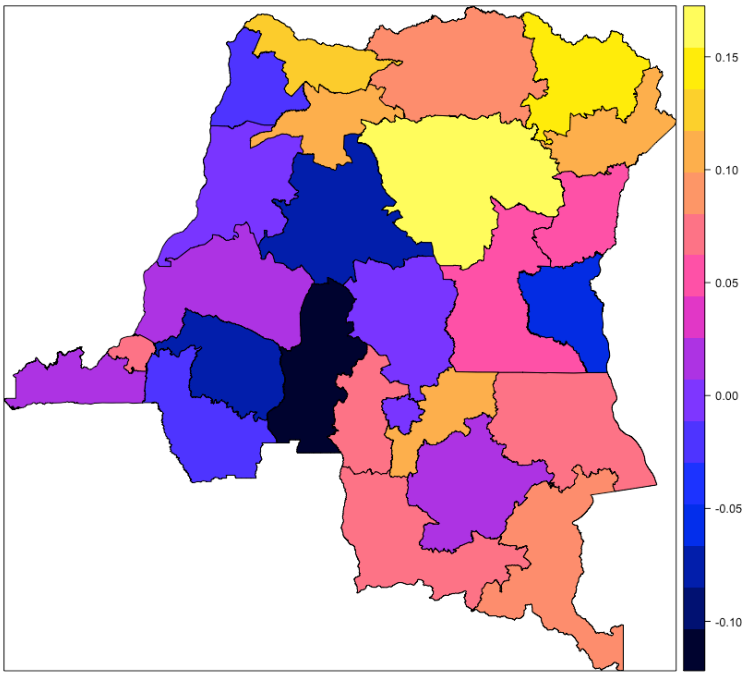
Supplementary Figure C1: Cluster-level prevalence estimates between kids (y-axis) and adults (x-axis)



Supplementary Figure C2: Cluster-level prevalence estimates between kids (y-axis) and adults (x-axis) – restricted to clusters with at least 10 adults and 10 children per cluster (n = 394 clusters).



Supplementary Figure C3: Map of outlier clusters (the adult prevalence is more than 25% higher than the prevalence amongst children) highlighted in orange. This map displays only clusters with at least 10 adults and 10 children.



Supplementary Figure C4: Prevalence differences between children and adults. Positive proportions represent provinces where prevalence is higher amongst children, negative proportions are provinces where prevalence is higher amongst adults.

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